Vol. 18 No. 3

March, 1952

THE BOTANICAL REVIEW

Interpreting Botanical Progress

Theories of the Biogenesis of Flavonoid Compounds. II.
T. A. GEISSMAN AND ELLY HINREINER 165

FUTURE CONTENTS
ON INSIDE BACK COVER

Published Monthly except August and September at
111 E. Chestnut St., Lancaster, Pa.

Entered as second-class mail matter February 4, 1935, at the Post Office at Lancaster, Pennsylvania, under the Act of March 3, 1879.

THE BOTANICAL REVIEW

Founded in 1935 by
HENRY ALLAN GLEASON and EDMUND H. FULLING
Managed, edited and published at
The New York Botanical Garden
by
EDMUND H. FULLING

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Articles are obtained primarily by invitation. Unsolicited manuscripts are also welcome. In all cases, however, the editor reserves the right to accept, reject or suggest revisions in submitted material. All manuscripts should be on double-space typewritten pages, with references and citations in accordance with the customary style of THE BOTANICAL REVIEW.

Missing numbers can not be supplied unless notice is received within two months after appearance of issues concerned.

All correspondence should be addressed to THE BOTANICAL REVIEW, at 111 E. Chestnut St., Lancaster, Pa., or Fordham P. O., New York, N. Y.

THE BOTANICAL REVIEW

VOL. XVIII MARCH, 1952 No. 3

THEORIES OF THE BIOGENESIS OF FLAVONOID COMPOUNDS (PART II)

(Concluded from February Issue)

T. A. GEISSMAN AND ELLY HINREINER

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Theories of Plant Synthesis 165
Existing Theories of Biosynthesis 165
Evidence from Genetic and Chemical Investigations 174
The Characteristic Structures of Flavonoid Compounds 183
The Formation of the Carbocyclic C_6-Fragments 185
The Attachment of the C_3-Side-Chain 187
The Nature of the C_6(B)-Precursor 188
The Hexose Precursor of the C_6-Units, and the Ring Closure 189
The Formation of C_6(B)-C_3 191
The C_6(A)-Fragment 194
The Oxidation Levels of C_3 197
The Oxidation Levels of C_3 in C_6-C_3-C_6-Compounds 199
Evidence from the Structures of the Isoflavones 203
Stilbenes and Xanthones 208
Benzophenones 210
Brazilin and Hematoxylin 212
Hydrangenol 214
The Attachment of Sugar residues 215
Origin of the Methoxy and Methylenedioxy Groups 217
Coumarins 223
Chromones 228
Isoprenoid Side-Chains, Furan- and Chromano-rings 229
Summary 233
Glossary 234
Literature Cited 237

THEORIES OF PLANT SYNTHESIS

EXISTING THEORIES OF BIOSYNTHESIS. Reference has already been made to theories of the biogenesis of plant substances other than the flavonoid compounds. It is not possible to review these proposals in detail; but because it is undoubtedly true that there are close relationships between the synthetic paths leading to compounds of widely diverse classes, it will not be possible to dismiss these related theories without further comment. The common feature of all present theories of plant synthesis is found in their

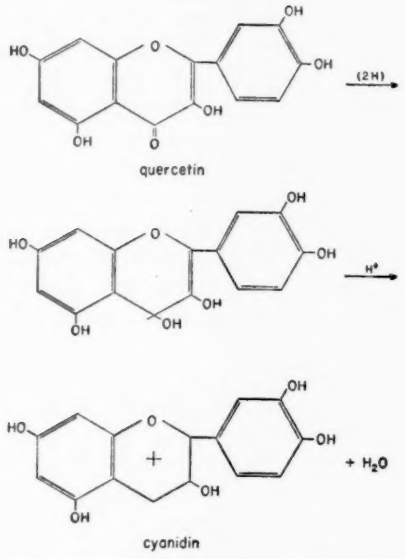
necessarily speculative nature. That more precise theories may be forthcoming is suggested by the inception of experimental approaches based upon an increasing application of the principles of enzyme action. Such studies as those of Thimann (Thimann and Edmondson, 1949; Edmondson and Thimann, 1950) and Heller (1948) on the biogenesis of anthocyanins represent this kind of attack.

The experimental basis of the existing theories consists largely in the isolation and structure proof of the compounds occurring in plants. The results of experiments designed to show that certain compounds which occur together in nature are interconvertible in the laboratory have often been advanced as evidence that such transformations can account for the coexistence of two or more related compounds in a single plant or a single species. The mere fact of such a coexistence, coupled with a structural relationship which suggests an in vivo interconversion by a plausible reaction, forms the basis for other theories. Reasoning of this kind is based upon what has been called the " comparative anatomy " of natural substances (Schöpf, 1934), and, while capable of modification in the direction of a greater degree of precision by experimental confirmation of some of the reactions proposed, represents the basis upon which nearly all existing theories of biogenesis rest.

The reverse process, in which the emphasis is placed upon the processes and starting materials known to be available in the cell, has so far resulted in no theory in which this approach is carried through to a conclusion with regard to any complex compound. In some cases early precursors can be defined, but the later stages remain obscure (Bonner, 1949; Calvin, 1949). In others, notably in the brilliant experiments dealing with biosynthesis in *Neurospora* (Beadle, 1949), it has been possible to define the nature of immediate precursors (e.g., 3-hydroxyanthranilic acid as the precursor of nicotinic acid (Mitchell and Nye, 1948)) of compounds already at a high level of complexity. So far as flavonoid compounds are concerned, there is little to say with regard to this " constructive " approach, such experiments as those of Thimann and Edmondson on anthocyanin formation not yet having been carried to the point where conclusions can be drawn as to actual synthetic mechanisms.

The " reconstructive " approach (Hall, 193[illegible]) to the course of plant biosynthesis has found expression in many theories. Emde

(1931), from the viewpoint of structural plausibility, has devised hypothetical syntheses for fatty acids, carotenoids and other substances. The suggestion has often been made that coexisting flavones and anthocyanins are related by the oxidation-reduction process (see Blank, 1947, p. 266):



(24)

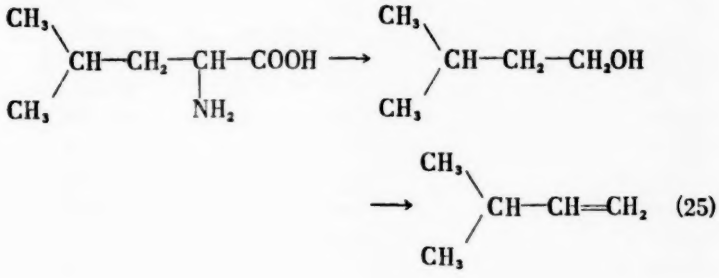
While Everest (1917) and Freudenberg (1925*a*, *b*) applied this idea chiefly to the unglycosylated compounds, Sando found support for his suggestion in his discovery of the coexistence (in the Jonathan apple) of the 3-galactosides of quercetin (217*d*) and cyanidin (270*b*) and the presence in brown-husked maize and purple-husked maize, respectively, of isoquercitrin (217*a*) and chrysanthemin (270*a*) (both of which are 3-glucosides), and was led to suppose that the conversion of the flavone into the anthocyanin constituted the final stage in the synthesis of the latter (Sando, Milner and Sherman, 1935; Sando, 1937). This theory found its earliest expression in the work of Wheldale (Wheldale and Bassett, 1913, 1914) on the chemistry and genetics of flower color in *Antirrhinum majus*. She observed that when white and ivory or white and yellow (flower-colored) plants were crossed, red or magenta offspring resulted, and suggested that the white

parent contributed a factor which converted the flavone of the ivory or yellow parent into anthocyanin. The flavones (as glycosides) isolated by Wheldale were apigenin (186) (from ivory) and luteolin (194) (from yellow, along with apigenin). The elucidation by Willstätter of the structures of the anthocyanins as 3-hydroxy compounds in the years immediately following Wheldale's work showed the untenability of her hypothesis, since luteolin and apigenin lack the 3-hydroxyl group. Onlsow (neé Wheldale) later (1925) suggested derivation of both the anthocyanins and flavones from some "parent" compound. Robinson (1936) has discarded the interconversion theory in the case of the flavone-anthocyanin pairs on the grounds that the coexistence of identically hydroxylated flavones and anthocyanins is the exception more often than the rule, and that when such a pair as observed by Sando is found side by side the compounds contain the 3′,4′-dihydroxy (e.g., as in quercetin, luteolin, cyanidin) grouping—the most common of the hydroxylation patterns (Lawrence et al., 1939). Indeed, the coexistence in the same plant of such a pair as isolated by Sando is unique and consequently does not furnish convincing evidence for the generality of the view that compounds of these kinds are synthetically related in such a simple and direct manner. Freudenberg (1925*a*) has discussed the relationship of the C_6-C_3-C_6-tannins to the flavonoid pigments, and has pointed out that (although exceptions are common) when compounds of different classes (e.g., a flavone and an anthocyanin) exist together in the same plant, they correspond in the positions of the phenolic hydroxyl groups. As examples are quoted the coexisting pairs homoeriodictyol (247) with chrysoeriol (196), delphinidin (272) (allegedly) with myricetin (231), cyanomaclurin with morin (222), quercetin (217) with catechin (267), etc. Again it must be pointed out that the exceptions are too numerous to enable one to regard such facts as compelling evidence of the direct interconversion of flavonoid compounds which differ only in the state of oxidation of the C_3-moiety by terminal oxidation or reduction processes affecting this portion of the molecule alone. It is probable that the attractiveness of this theory stems in part from the classical experiment of Willstätter (Willstätter and Mallison, 1914), in which the reduction of quercetin (pentamethyl ether) led to authentic cyanidin chloride, and Freudenberg's (1925*b*) reduc-

tion of cyanidin to epicatechin. The conversion of flavonols into anthocyanidins by chemical means is by no means a smooth process (Willstätter and Mallison, 1914; Geissman and Clinton, 1946; Asahina et al., 1928, 1929; Kondo, 1932; Kondo and Segawa, 1932) but leads to a mixture of substances in which the authentic anthocyanidin is present, if at all, in relatively small amount; and the catalytic hydrogenation of an anthocyanidin to a catechin can hardly serve as an acceptable model for a cellular process.

Hall (1937) has adopted the reconstructive approach in devising a theory of biogenesis dealing in particular with the C_6-C_3-group of substances, but applied to many other classes of plant constituents. The theory depends upon assumptions concerning the fundamental role of metasaccharonic acid in giving rise to a "parent" C_6-C_3-unit which is capable of further transformation along many paths. The theory is speculative in the extreme and succeeds in devising routes of synthesis only by the arbitrary selection of appropriate intermediates and arbitrary assumptions concerning the mode of combination of the reactants. There appears to be little experimental validity, in terms of cellular processes, for many of the reactions assumed. In an earlier paper (Hall, 1933), an equally broad theory of biogenesis of terpenes is developed. In the light of modern ideas on the mechanism of organic reactions, as well as present-day knowledge of plant and other biochemistry, these theories appear to lack the realism which is needed to render even speculative proposals attractive starting points for further development.

Frey-Wyssling (1938) has put forward a general theory of the origin of secondary plant constituents which is based upon (*a*) the degradation of α-amino acids to olefins, typified by the following changes:

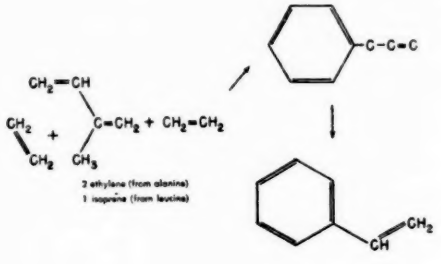


(25)

(*b*) the combination of olefinic molecules, obtained in this way or by further dehydrogenation of the basic olefins (e.g., isoamylene → isoprene), into more complex hydrocarbons, and (*c*) the oxidation (hydroxylation, dehydrogenation, side-chain degradation, etc.) of such unsaturated hydrocarbons to the final oxygenated substances.

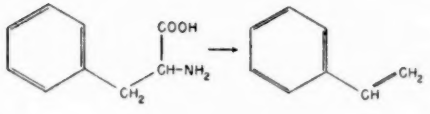
As an example of the application of this scheme, the genesis of the flavonoid skeleton could be represented as follows:

(a)



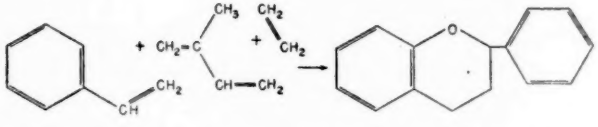
(26)

or, (b)



(27)

(c),



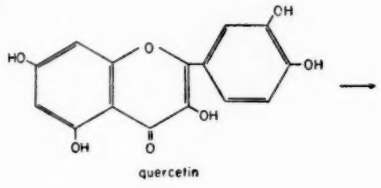
(28)

Many of the reactions postulated in this theory lack convincing biological analogy. Moreover, while olefins are known to occur (but to a very limited extent, except for the terpenes themselves) in plants, it seems far more plausible to consider them as being derived from, perhaps in the general way suggested by Frey-Wyssling, rather than as giving rise to, substances which are themselves the true precursors of the complex secondary plant substances. The theory of Frey-Wyssling resembles in certain aspects some of the proposals of Robinson in his general theory of alkaloid biogenesis, but goes beyond the Robinson theory by departures in

directions which cause it to leave the relatively firm footing of recognized biochemical analogy. Onslow (1931) had earlier suggested that the deamination of amino acids might give rise to substances which could undergo condensation to form flower pigments. Although both Onslow's and Frey-Wyssling's hypotheses were advanced before the relationship of the amino acids to the other participants in cellular metabolism was known or fully recognized, it appears highly likely that the role these authors suggest for the amino acids, considered broadly and not in detail, is a correct one. It is also apparent that, with the advances which have been made in understanding the cellular metabolism of plants and animals, it is now the details of the synthetic processes which need to be worked out. While it is known that olefins can combine, for example, by polymerization and by reactions of the Diels-Alder type, the combinations pictured by Frey-Wyssling have a degree of specificity which could be explained only by the supposition that they are enzyme-controlled and that the enzymes which direct these condensations cause them to proceed in ways which are not in the direction in which a non-enzymatic reaction would be expected to occur. For the most part, enzymes appear to act in such a way that the reactions they mediate proceed in a manner dependent primarily upon the chemical and structural properties of the reactive molecule or molecules, the enzyme serving chiefly to bring about the normal reaction, based upon predictable structural factors, at a useful rate. A high degree of coincidence is required to accommodate the view that enzymes exist which can bring about the union of olefinic reactants and at the same time do this in a way which is often different from that which would be predicted on the basis of present-day concepts of reaction mechanisms.

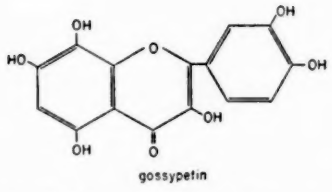
Seshadri, whose work in the chemistry of naturally-occurring substances has resulted in many valuable contributions to our knowledge of the flavone constituents of plants, has summed up the implications of the results of his work upon plant biogenesis in several papers (Rao and Seshadri, 1943; Seshadri, 1948, 1949). The discovery that the hydroxylation of flavones by means of potassium persulfate makes possible the interconversion of many natural flavones into others, also naturally-occurring, has led Seshadri to speculate upon the biogenesis of these substances by the addition (and removal) of hydroxyl groups. The process is

visualized as simulating the in vitro oxidation and is supposed to occur at a stage in the synthesis at which one fully aromatized flavone acts as the immediate precursor of another. An example is the following:



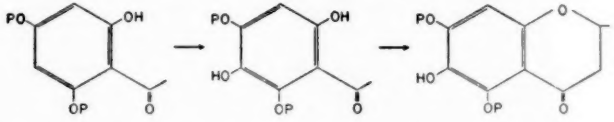
quercetin

(29)



gossypetin

The alternative possibility—that oxidation occurs before closure of the heterocyclic ring—is represented by the partial structures

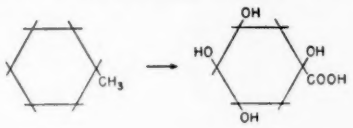


(30)

where P is a "protective" group. The assumption of the presence of a protective group is considered to be necessary, since the oxidation, when carried out in the laboratory, is not successful when a number of free hydroxyl groups are present. This difficulty is commonly overcome by working with methyl ethers, followed by a final step in which the methoxyl groups are converted into hydroxyl groups. Since it is unlikely that methylation-demethylation as a measure for affording such "protection" occurs in the cell, it is supposed that the protective mechanism is one of "absorption or chemisorption" on the enzymes required for the oxidation step. This appears to be another way of saying that the enzymatic oxidation has a structural specificity such that the

presence of a number of hydroxyl groups on the molecule does not labilize the nucleus to attack at more than one position.

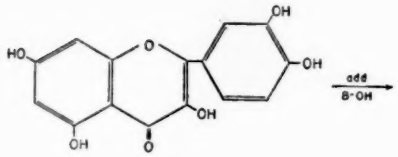
The introduction of hydroxyl groups into aromatic and heterocyclic nuclei in the so-called "detoxication" of compounds such as aromatic hydrocarbons, quinoline derivatives, etc. (Williams, 1947) in the animal organism offers a basis for the inference that plant cells may contain enzyme systems capable of carrying out analogous reactions. However, the extent to which such hydroxylation reactions can and do occur in plants is not known; and there is no known analogy to support Frey-Wyssling's suggestion that so extensive a process of hydroxylation as the conversion of methylcyclohexane (X) to quinic acid (XI) is possible:



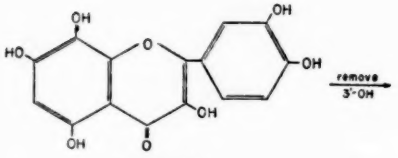
X XI (31)

The presence of phenol oxidases throughout the higher plants is well recognized: examples are the ubiquitous tyrosinase, laccase and the related ascorbic acid oxidase. The typical reaction of these enzymes is the conversion of an *o*-dihydroxy benzene derivative (e.g., catechol) into the corresponding quinone (polyphenol oxidase action), but the reaction of present interest—monophenol oxidase activity—is of limited distribution, being associated chiefly with tyrosinase (or a monophenol oxidase component of this possibly non-homogeneous enzyme). It would appear that the numerous and highly specific hydroxylations of Seshadri's scheme of biogenesis must be supposed to depend upon an oxidase or oxidases of which tyrosinase is a model.

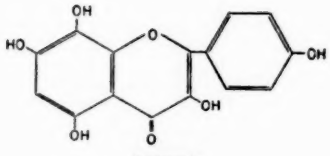
A second kind of reaction suggested by Seshadri is the removal of an aromatic hydroxyl group. In the general theory, quercetin occupies a central role, chiefly because of its great predominance among the naturally-occurring flavones. Consequently, in those plants which contain quercetin along with another flavone, the latter is regarded as being derived from the former. The co-occurrence in *Gossypium herbaceum* of quercetin, herbacetin and gossypetin leads to the suggestion that the genesis of the latter two from quercetin follows the course:



quercetin



gossypetin (32)



herbacetin

The removal of the aromatic (3'-) hydroxyl group is without analogy either in classical organic chemistry or in biological reactions, and the need for assuming such an unlikely reaction is a serious defect in the theory. While it could be argued that gossypetin may be formed from quercetin in the suggested manner, herbacetin being derived in another way—perhaps arising from a precursor in common with quercetin—it must be pointed out that if the presence of a precursor common to quercetin and herbacetin must be inferred, there appears to be no compelling reason for discarding the still simpler possibility that a single precursor is common to quercetin, herbacetin and gossypetin.

EVIDENCE FROM GENETIC AND CHEMICAL INVESTIGATIONS. A line of inquiry more fruitful than those so far discussed is that in which reconstructive reasoning is associated with experimental evidence that at least some of the transformations supposed to occur in the cell can be duplicated in the laboratory under "physiological conditions" of temperature and pH, from starting materials which are known or may reasonably be supposed to occur in the

plant, and in the absence of special reagents which are not likely to occur in the plant. Theories of plant biosynthesis based upon this approach are best exemplified by those dealing with alkaloid biogenesis. " Constructive " reasoning of this kind has met with conspicuous success in the field of alkaloids, leading not only to the postulation of in vivo reactions which appear to have a high degree of probability, but also to the prediction of structures of complex alkaloids in advance of their final elucidation by the methods of organic chemistry and to the actual synthesis of alkaloids under " physiological conditions " from starting materials of known or highly probable existence in plants.

Kindred approaches to the problems of the biogenesis of non-nitrogenous plant constituents have not reached a comparable stage of development. The initial suggestions of Robinson still constitute the most plausible starting point from which to introduce the elaborations which are permissible on the basis of recent advances in the understanding of plant biochemistry, cellular metabolism and the genetic control of plant constituents.

It has been suggested in this review and elsewhere (Geissman, 1949*b*) that an enzyme-mediated reaction is best regarded, unless evidence is found to the contrary, as proceeding by a route predictable on structural grounds, and that the role of the enzyme is to catalyze a transformation which occurs by way of a " normal " mechanism. In short, since the reactions which are being dealt with are those of organic compounds, they probably proceed according to the recognized principles governing organic reactions. The justification for attempting to devise a hypothetical sequence of reactions to account for synthesis in a plant is primarily that such a scheme may offer points of attack by direct experimental methods. Such a hypothetical scheme should be therefore based upon sound and admissible assumptions. Some of these assumptions—relating to the choice of starting materials, intermediates and available synthetic mechanisms—have already been discussed in detail. Many of the theories discussed in the earlier part of this section suffer from the defect that they embody reactions which are not only without sound biochemical analogy, but would not be expected to proceed under any conditions other than under the influence of a highly specific enzyme capable of catalyzing not only a unique reaction (which is common), but a unique kind of reaction (which is rare).

One of the most fruitful areas of study from which to draw information concerning biosynthesis is that of chemical genetics—the study of the interrelationship between the chemical constitution of an organism and genetic factors. The earliest investigations in this field are those of Wheldale and Bassett (1913, 1914), whose pioneering studies of the chemical constitution of known genotypes of *Antirrhinum majus* have in recent years been extended both in scope and in detail by many other investigators (see Lawrence, 1950).

These investigations have dealt almost exclusively with the anthocyanin flower pigments, and the results that have been obtained show clearly that the formation and chemical structure of these pigments are under genetic control. It has been found that specific genes may control the following:

a) the presence or absence of anthocyanin pigment,
b) the concentration of anthocyanin in the cell sap,
c) the degree of oxidation (i.e., the number of hydroxyl groups) in the anthocyanidin,
d) the extent to which methylation of the anthocyanidin hydroxyl groups occurs,
e) the number and position of sugar residues in the anthocyanin,
f) the pH of the cell sap,
g) the presence or absence of anthoxanthin co-pigments ("blueing factors") in the cell sap.

In addition, there is scanty evidence (Geissman and Mehlquist, 1947; Geissman and Bockian, 1950) which suggests that equally specific control of the non-anthocyanin petal constituents may be demonstrated when these substances are subjected to a more careful examination than has so far been possible. The important conclusion emerges from these studies that single genes control simple chemical differences. A more explicit exposition of this principle is found in the studies of Beadle et al. (1949) on the genetic control of the chemical reactions involved in the metabolism of *Neurospora*. It has been demonstrated there that single genes control not only simple biochemical differences but single chemical reactions. It may be taken as a provisional hypothesis that this will be found to be true of higher organisms, and consequently that a gene for a given flower color variation controls one specific chemical reaction at some stage in the probably complicated se-

quence of steps by means of which the final complex pigment is produced from simple starting materials.

Existing theories of pigment biosynthesis which are based upon genetic data antedate the advances in chemical genetics which have been made in recent years. For this reason certain of the quantitative conclusions drawn from studies on the genetics of flower coloration are probably less reliable than they may at first have appeared to be, although the qualitative aspects of these investigations are, for the most part, acceptable. A detailed and critical analysis of the existing data and theories does not appear to be justified at the present time, for several reasons. For one thing, it is probable that in some of the investigations all of the necessary chemical data are not available (cf. Bate-Smith, 1949). If attention is centered upon the anthocyanin pigments of a series of color variations of a given flower, and only casual scrutiny given to the acyanic pigments, the picture must be quite incomplete with regard to the biosynthetic process, although possibly nearly complete with regard to specific structural changes in or near the stage of some of the end products. For example, if the dominant allele of a gene is known to be responsible for the production of an anthocyanin containing the 3′,4′-dihydroxyphenyl nucleus, its recessive form producing the anthocyanin possessing the 4′-hydroxyphenyl group, it cannot be said with certainty at which stage the gene (or enzyme) acts unless definite information concerning both the structure of the accompanying flavone derivatives and the biosynthetic relationship between these and the anthocyanins is at hand.

Recent investigations (Seikel and Geissman, 1951) on known *Antirrhinum majus* genotypes have led to results which raise further serious questions regarding previous genetico-chemical studies on the flower pigments of this plant. Earlier work (Wheldale and Bassett, 1913, 1914; Scott-Moncrieff, 1930*a*, *b*) showed the presence of luteolin (194), apigenin (186), cyanidin (270) and pelargonidin (269) in *A. majus* color variations. Recently, a careful re-examination of genetically analyzed *Antirrhinum* blossoms has been started, and preliminary work on miscellaneous yellow (ppMMyy, ppMmyy, ppmmyy) flowers has disclosed the presence of a benzalcoumaranone glucoside (aureusin), a mixture of flavone glycosides, and a flavanone glycoside (naringenin-?-glucoside). The flavone mixture appeared to contain both luteolin (194) and

apigenin (186). It is significant that when a pure yellow genotype (ppmmyy) was studied, a pure apigenin glucoside was readily isolated. These data, although incomplete, suggest that the M factor in the ppM-yy (yellow) flowers (present among the "mixed" yellow flowers) is responsible for luteolin formation, its recessive (mm) giving apigenin only. In the early work of Wheldale and Bassett, referred to above, the yellow flowers from which luteolin and apigenin were isolated must have been a mixture of phenotypically identical genotypes, differing in the factors (M) (m). These findings emphasize the caution that must be observed in drawing inferences concerning the chemical composition of one genotype on the basis of experimental results secured with another.

A detailed examination of color variations of the carnation (Mehlquist and Geissman, 1947; Geissman and Mehlquist, 1947) has disclosed that yellow and white flowers may contain the same flavone (kampferol, 206); it is doubtful that this would have been ascertained by any approximate methods of testing.

The results of the detailed and careful studies on the flower pigmentation in *Dahlia variabilis* have led Lawrence and Scott-Moncrieff (1935) to propose a theory of specific pigmentation (see Scott-Moncrieff, 1937). In this plant, as well as in *Papaver Rhoeas* (Scott-Moncrieff, 1939), suppression effects of one gene upon another have been observed, suggesting that genes may compete for a limited source of a common presursor. In the theory of Lawrence and Scott-Moncrieff (1935) genes appear to possess the dual roles of making available the common source material and of elaborating this source material into the final pigment. This is difficult to reconcile with the present-day view that genes bear a one-to-one relationship to enzymes and that given enzymes have single (or at least very limited) functions in a given cellular organization. While objections may be raised to the details of the theory of Lawrence and Scott-Moncrieff, the evidence appears to support in a qualitative sense the position taken by these authors. However, it must be pointed out that even in the qualitative sense their hypothesis based upon genetico-chemical studies must be confined to the plants upon which they are based. That is to say, it is not necessarily true (and Lawrence and Scott-Moncrieff do not state) that, if anthocyanin and flavone pigments are derived from

a precursor common to both, this precursor is always so limited in amount that an increase in the production of the anthocyanin will result in a drop in flavone production. It was found in the carnation (*Dianthus caryophyllus*), for example, that a crimson and a white flower had the same flavone concentration (Geissman and Bockian, 1950). It has also been found (Geissman and Mehlquist, 1947) that in a series of three scarlet forms, differing only in their content of an " inhibitor " gene, and in which the anthocyanin pigment concentrations were in the ratio 1 : 2 : 4, there is no obvious " blueing " effect in the least-deeply colored. A strict interdependence of anthocyanin : flavone concentration would suggest that the anthocyanin : flavone ratio in such forms would vary as much as 16-fold (1/4 : 2/2 : 4/1), a change which should modify not only the intensity but also the shade of the petal colors. The observation is that only the intensity shows a variation, no " blueing " effect being noticed in the flowers having the lowest anthocyanin content.

It is the conclusion of the reviewers that theories of flower pigment biosynthesis based upon experimental work in which the structures and concentrations of the acyanic flavonoid pigments have been ascertained only by approximate and qualitative methods are to be accepted with reservations only. Because of the importance of the non-anthocyanin pigments, it is unfortunate that the experimental difficulties of determining their structures and concentrations in small amounts of plant material are still so great. It is to be hoped that application of the new techniques of chromatography, polarography and spectrometry may aid in the solution of this important problem.

Robinson has discussed the formation of anthocyanins in plants (1936), pointing out that the only generalization that may be considered to be established is that the pigments are formed from simple carbohydrates. This conclusion rests in part upon the observations that anthocyanins are frequently produced at sites of intense photosynthetic activity (rapidly growing shoots), when translocation of photosynthetic products is impeded (autumn coloration, injury), and when abundant nutrients (sugars) are artificially fed; Blank (1947; pp. 270–281) has discussed these points in detail. Since the simple sugars may, from the present viewpoint, be con-

sidered equivalent to the many simpler substances to which they are related through the anabolic (including photosynthesis) and catabolic (sugar breakdown) processes of the cell, the generalization may be made that the anthocyanins are formed from the simple sugars or their breakdown products.

This generalization is almost obvious, considered in its broadest sense; but when examined in detail it soon becomes apparent that it serves as a logical and fruitful starting point for considering the details of the biosynthetic processes. Moreover, while Robinson had particular reference to the anthocyanin pigments, there is no reason to exclude other flavonoid (and related) substances from consideration.

When all of the flavonoid compounds are considered, the range of oxidation and reduction encountered in nature is wide, extending from a degree of reduction represented by flavone, $C_{15}H_{10}O_2$ ($C_{15}H_{10}O_5$ = carbohydrate oxidation level), to the oxidation level found in nor-gardenin, $C_{15}H_{10}O_9$. If the source of substances of this class is indeed the carbohydrates of the plant, it is apparent that there exist in the living cells oxidation and reduction systems capable of a wide range of activity.

Robinson has pointed out that the state of oxidation of pelargonidin is that of normal carbohydrates, cyanidin and delphinidin requiring oxidation above this level. This relationship can be expressed in the following equations:

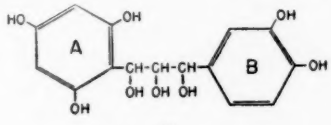
(a) 2 hexose + 1 triose = $2(C_6H_{12}O_6)$
$+ C_3H_6O_3 = C_{15}H_{30}O_{15}$ (33)

(b) $C_{15}H_{30}O_{15} - 9\,H_2O = C_{15}H_{12}O_6$ (= pelargonidin). (34)

Thus it is apparent that the (formal) derivation of pelargonidin from carbohydrate sources requires only the removal of the elements of water, but no overall oxidation or reduction. It seems likely, as Robinson (1936) suggested and as the discussion in a later part of this section will indicate, that, although pelargonidin is at the carbohydrate level of oxidation, this level is attained by way of steps which include a reduction and an oxidation. Whether the reduction stage involves removal of the 3′-hydroxyl group in Robinson's "normal" precursor, or occurs at some highly elaborated (non-aromatic) level, cannot be said with assurance, but

the latter alternative will be given the greater weight in the discussion to follow.

Robinson suggests that the " normal "hypothetical intermediate in anthocyanin synthesis in the plant is (XII):



XII

$C_{15}H_{16}O_8$

which is at the oxidation level of the carbohydrate precursors:

$$(C_{15}H_{30}O_{15}) - (7\ H_2O) = C_{15}H_{16}O_8 \qquad (35)$$

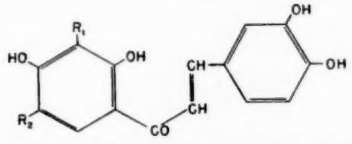
The additional suggestion that the photosynthetic process consists in a condensation of $C_6(B)$-C_3 with $C_6(A)$ appeared to be supported by the results of genetic studies, particularly on *Dahlia,* since it appeared that all of the pigments present in *Dahlia*—cyanidin, pelargonidin, apigenin and the unknown " yellow " flavone—were phlorglucinol derivatives ($C_6(A)$), differing only in the degree of oxidation of the $C_6(B)$-C_3-fragment. Since the subsequent demonstration (Price, 1939) that the yellow pigment is, at least in some cases, the chalcone butein (258), the $C_6(A)$-portion of which is a dihydroxy moiety, it now seems unlikely that the " common "precursor in *Dahlia* is XII.

Evidence from studies on the constituents of the flowers of *Coreopsis* species and of related Coreopsidinae suggests the hypothesis that the flavonoid compounds arise by a condensation of a common $C_6(B)$-C_3-fragment with $C_6(A)$-fragments of varying constitution, or with a common $C_6(A)$-fragment which can be later modified in more than one way during the process of aromatization. The genetic relationship between the various members of the Coreopsidinae may be inferred from their taxonomic relationship. There can be little doubt that these plants possess many genetic factors in common (Sturtevant, 1948). Certain of these genes find expression in the morphological similarities upon which the taxonomic classification of the sub-tribe is based, and it is a permissible inference that these related genera possess additional genes in common which do not find obvious phenotypic expression.

Among these genes are probably those which are responsible for the striking chemical similarities which have been discovered in the study of their flower pigmentation.

Four species of *Coreopsis* and one of *Cosmos*, all having yellow to orange-yellow rays, have been examined with particular regard to the pigment responsible for the color change from yellow to red when the petal is treated with dilute alkali. All of these flowers contain chalcones, and in addition a pigment of one of them possesses the unique benzalcoumaranone structure.

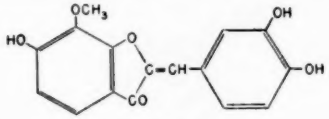
Coreopsidinae flower pigments



$R_1 = R_2 = H$: butein (*Coreopsis Douglasii, Coreopsis gigantea, Cosmos sulfureus, Dahlia variabilis*)

$R_1 = OCH_3$, $R_2 = H$: (*Coreopsis grandiflora*)

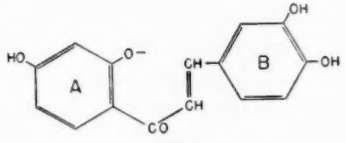
$R_1 = H$, $R_2 = OH$: stillopsidin (*Coreopsis Stillmanii*)



leptosidin (*Coreopsis grandiflora*).

Hattori (1950) has also found glycosides of butein and leptosidin in the rays of *Coreopsis lanceolata* and *C. saxicola*. In addition to butein, luteolin and quercetin were found in *Cosmos sulfureus*.

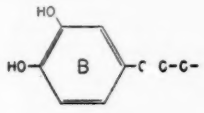
It is significant that all of the pigments so far recognized in the *Coreopsis* and *Cosmos* species which have been examined contain the grouping XIII:



XIII

and differ from each other in the degree and position of further hydroxylation in the A-ring and in the degree and nature of oxi-

dation of the C_3-fragment. These observations support the theory that the structural unit XIV



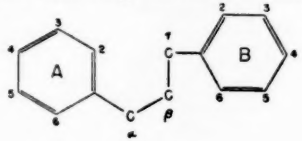
XIV

arises in these plants as a result of processes which are under the control of certain genes common to (but because of the ubiquity of this unit, not unique to) the Coreopsidinae. Whether the $C_6(B)$-C_3-unit is preformed, the ring being aromatized prior to the condensation with the fragment which will eventually become $C_6(A)$; or whether the catechol nucleus present as $C_6(B)$ is elaborated after the C_6-C_3-C_6 condensation has been completed is a question of fundamental importance, and one not easily answered. There appears to be some reason to incline toward the former view, since *Dahlia* contains pigments (apigenin and pelargonidin) having a 4-hydroxyphenyl group as the $C_6(B)$-component, a fact which suggests that a precursor exists which gives rise to both 4′-OH and 3′,4′-diOH $C_6(B)$-C_3 units. This question will be examined in detail in a later section of this review.

Questions such as those raised in the preceding paragraph are the kind which a satisfactory theory of plant biosynthesis must deal with in the attempt to avoid arriving at too facile a conclusion. The theories of biosynthesis already discussed have often dealt inadequately with essential details largely because they were devised before phytochemical, biochemical and genetic studies had progressed to a sufficient degree to furnish the necessary factual and conceptual basis upon which to formulate a theory which did not violate what is now known to be true or held to be valid. In the ensuing portion of this review an attempt will be made to select from the theories of biosynthesis of the past that which appears still to be valid, and to supplement them with sufficient evidence from more recent advances to build a theory of plant biogenesis which is plausible from the standpoint of the present state of our knowledge.

THE CHARACTERISTIC STRUCTURES OF FLAVONOID COMPOUNDS. It will be helpful to summarize at this point the most common

structural features of the naturally-occurring flavonoid substances. With reference to the following fundamental nucleus * (XV),



XV

the following generalization may be made:

a) The greatest number of compounds are hydroxylated † at B-3,4, with B-4 and B-3,4,5 also common. Hydroxylation at B-2 is known, but no example is known of B-2,6 hydroxylation.

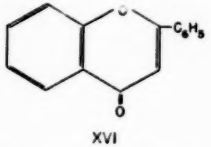
b) Hydroxylation is always found at A-2, and in the great majority of cases, at A-2,4,6. With a few exceptions, hydroxylation always occurs at least at both A-2 and A-4.

c) Hydroxylation is known to occur at all possible positions A; flavones are known which are A-2,3,4,5,6-hydroxylated.

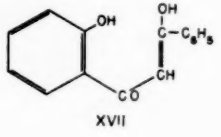
d) The B-4 hydroxyl group in the anthocyanins is always free, never occurring in the glycosylated or methylated form.

e) With the exception of the A-5 and B-2 positions, sugar residues have been found attached to any hydroxyl group in either ring, but are most commonly found at the A-4 and the C_3-β hydroxyl groups.

f) Sugar residues are found only at the C_3-β or at both the C_3-β and the A-6 hydroxyl groups of the anthocyanins.



XVI



XVII

g) The C_3-α carbon atom is in most cases present as a carbonyl group, but the state of oxidation of the C_3-chain may vary from

* Since flavones and other flavane derivatives are numbered differently from the open chain (in C_3) chalcones, etc., this arbitrary numbering is used at this point.

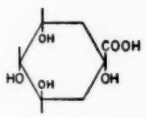
† The term "hydroxylated" is intended to include the heterocyclic oxygen atom in the chromane or chromone ring of flavone derivatives; for example, flavone (XVI) may be regarded for present purposes as being equivalent to the compound (XVII):

$$\text{A-}\underset{\alpha}{CH_2}\text{-}\underset{\beta}{CHOH}\text{-}\underset{\gamma}{CHOH}\text{-B to A-}\underset{\alpha}{CO}\text{-}\underset{\beta}{CO}\text{-}\underset{\gamma}{CHOH}\text{-B.*}$$

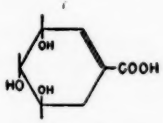
h) The kind and position of hydroxylation in the naturally-occurring C_6-C_3 compounds is similar to that found in the C_3-C_6(B)-fragment of the naturally-occurring flavonoids, and is usually quite different from that of the C_6(A)-C_3-fragments.

FORMATION OF THE CARBOCYCLIC C_6-FRAGMENTS. The origin of the C_6-fragments in the C_6-C_3-C_6-skeleton from carbohydrate (hexose) precursors, as originally proposed by Robinson, has been accepted either explicitly or implicitly by many investigators. Fischer's treatment of this idea has been discussed, and Hall's use of hexoses and hexonic acids having structures derived, frequently with extensive modifications, from the simple hexoses has been referred to. While Fischer has described a detailed (hypothetical) mechanism by means of which meso-inositol is formed from D-glucose, only an arbitrary sequence of steps can be devised to account for a subsequent attachment of a C_3-fragment to the inositol ring (to form C_6-C_3) or for the attachment to the inositol of C_6-C_3 to form the flavonoid skeleton. However, the inositol-hexose relationship can be utilized to advantage to make possible a conclusion concerning the point of attachment of C_3 to the C_6-residue.

The link between the inositols and the C_6-C_3 and flavonoid compounds appears to be the related pair of cyclitol carboxylic acids, quinic and shikimic acids. Quinic acid occurs as such in cinchona bark, coffee, bilberry, sugar beet, and as the 3-caffoyl ester (caffeic acid = 3,4-dihydroxycinnamic acid) in several other plants. Shikimic acid is configurationally identical with quinic acid in the 3,4,5-positions:

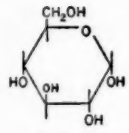


quinic acid

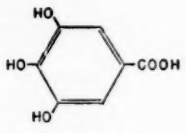


shikimic acid

* The abbreviated expression A-C-C-C-B will frequently be used to represent the C_{15} nucleus, in which A and B are six-membered carbocyclic rings.

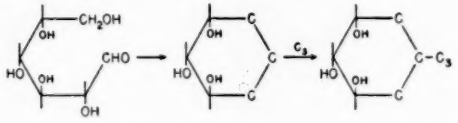


α-D-glucose



gallic acid

The striking configurational relationship between quinic and shikimic acids and D-glucose suggests a biogenetic relationship between these substances and between them and gallic acid (Fischer and Dangschat, 1935; Dangschat and Fischer, 1938). A further relationship to other plant substances is indicated by the ubiquity of the 3,4,5-trihydroxyphenyl nucleus (and methyl ethers of this) in a variety of naturally-occurring compounds: delphinidin (271), myricetin (230), gardenin (233), syringyl alcohol (27), angiosperm lignin, the mescal alkaloids, etc. In these substances, as in gallic acid, the point of attachment of the side chain is in the 1-position with respect to the 3,4,5-hydroxylation. These observations suggest that in the attachment of the side-chain (C_3 or C_6-C_3) to the carbocyclic fragment derived from the hexose, the point of attachment is at the carbon atom corresponding to the number one carbon atom of glucose: *

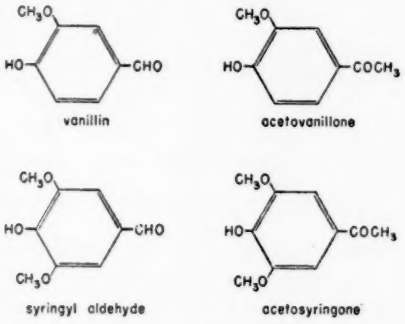


(35)

Gallic acid could arise either by the introduction of carbon dioxide into the above six-carbon cycle by a process similar to that involved in β-carboxylation of α-keto acids; or by the degradation by oxidative process of a C_3-side-chain. Of these, the latter course appears the more likely. This choice is made chiefly because of the existence in plants of a large number of compounds containing side-chains of one and two carbon atoms attached to C_6-residues possessing hydroxylation patterns characteristic of the common C_6-C_3-compounds. Hibbert (1941) has shown that degradation methods, based upon the use of alkali applied to certain lignins

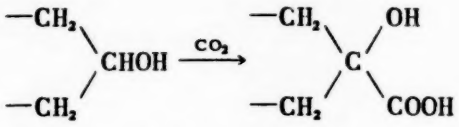
* When "partial" structures are used, as in this example, the nature and configuration of the constituents which are not shown are omitted in order that attention may be directed to those features under immediate discussion.

from both soft- and hardwood sources, leads to the isolation of C_6-C_2- and C_6-C_1-compounds such as vanillin, acetovanillone, syringyl aldehyde and acetosyringone.



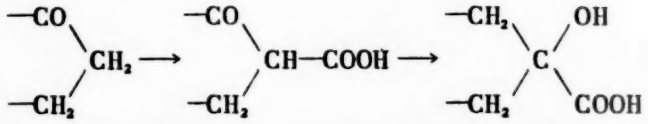
Hibbert concludes that these results establish definitely the existence of two-carbon side-chains in at least some of the spruce and maple lignin building units. The existence of C_6-C_3 building units in lignin molecules has been demonstrated by numerous investigators. It is significant that all of the C_6-C_1-, C_6-C_2- and C_6-C_3-units isolated by the breakdown of lignins possess a vanillyl or syringyl nucleus as the C_6 portion. Thus, while the C_6-C_1- and C_6-C_2-units could be considered to be formed by independent processes, their derivation from C_6-C_3-parents appears, for reasons to be presented in a subsequent section, to be the most likely assumption.

ATTACHMENT OF THE C_3-SIDE-CHAIN. The widespread occurrence of quinic acid and the cyclitols ("inositols") permits the inference that the synthesis of aromatic nuclei proceeds through cyclohexane derivatives, rather than the reverse, since the energy requirements for the reduction of, for example, gallic acid to quinic acid, would be high because of the considerable stability of the benzenoid ring. In the preceding section reasons were given which support the probability that quinic acid is derived from a hypothetical C_6-C_3-precursor. An additional argument for this assumption is found in the presence of the hydroxyl group on the carbon atom to which the carboxyl group is attached. It is biochemically improbable that a carboxylation of the type



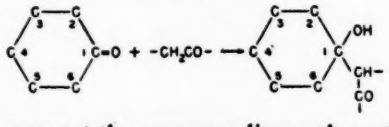
(36)

occurs in the plant, and a process such as



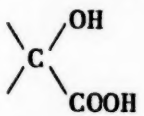
(37)

(in which the first step is plausible) involves a number of reactions whose choice must be highly arbitrary. If, however, the initial condensation of the hexose-derived C_6-moiety and a triose takes place at a carbonyl group in the carbocyclic component, the following (partial) reaction can be written:



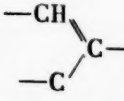
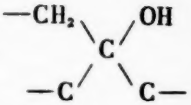
(38)

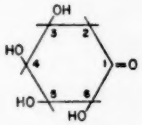
The numbers represent the corresponding carbon atoms of glucose. Degradation of the side-chain to a carboxyl group leads to the



grouping of quinic acid; modification of the side chain in other ways leads to substances of the C_6-C_3-type and, by a further C_6-condensation, to the flavonoid compounds.

THE NATURE OF THE C_6(B) PRECURSOR. The correspondence of the C_6-C_3-compounds found in nature (Tables 1 through 4) and the C_3-C_6-moiety of the flavonoid substances is, as Robinson originally suggested, highly probable. By far the greater number of C_6-C_3-C_6-compounds contain the 3′,4′-dihydroxy grouping (flavone numbering), and this structure predominates in the C_6-group. The 3′,4′,5′-trihydroxy grouping, as in delphinidin, myricetin,

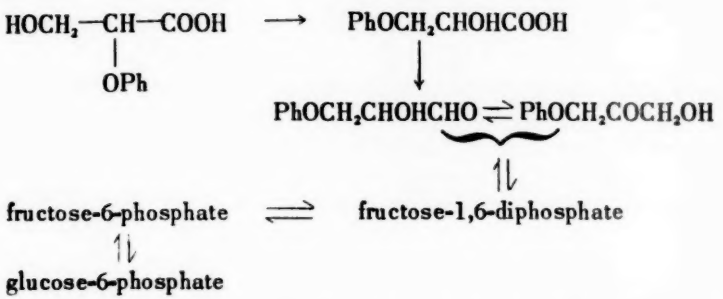
syringyl alcohol, etc., is also widely distributed. Other variants of the C_6(B)-nucleus, including those with no or one (para) hydroxyl group, and those with as many as four (as in apiole, etc.) are rare; but it is significant to observe that the aromatic nuclei of the C_6-C_3-compounds and the C_6(B)-nuclei of flavonoid compounds do not contain two hydroxyl groups ortho to the point of attachment of the side chain. The C_6(A)-fragments of the flavonoid compounds almost always contain two such ortho hydroxyl groups. The invariable presence of the  structure in the C_6(B)-C_3-type of fragments therefore supports the hypothesis that the  unit, found unaltered in quinic acid, is present in the precursor for the C_6(B)-C_3-unit of the flavonoids and for the corresponding C_6-C_3-substances. The inference is now strong that the hexose-derived C_6-unit to which the side chain becomes attached is XVIII



XVIII

where the numbers again refer to the carbon atoms of the original hexose.

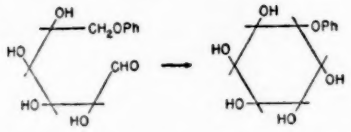
THE HEXOSE PRECURSOR OF THE C_6-UNITS, AND THE RING CLOSURE. It has been found (Calvin, 1949) that the early products of photosynthetic carbon fixation in green plants are the simple phosphorylated trioses, the products of the glycolytic processes, and the mono- and diphosphorylated hexoses. The suggested synthetic scheme is as follows:



Although glucose-1-phosphate does not appear in this scheme, its close relationship to glucose-6-phosphate in glycogen and starch metabolism permits its inclusion by way of the additional equilibrium:

$$\text{glucose-6-phosphate} \rightleftharpoons \text{glucose-1-phosphate} \qquad (39)$$

These substances, then, may be considered to be the starting materials for the elaboration of the two C_6- and the C_3-residues. The conditions which will serve as a guide in devising the most probable way in which the ring is formed have been outlined, and it was concluded that C_6(B) most probably arises by way of XVIII. As a first approximation, the formation of this unit from a hexose is best regarded as proceeding through either an aldol condensation or a nucleophilic displacement on a carbon atom bearing a phosphorylated hydroxyl group. Since ring closure by aldol condensation requires a —CH— group activated in such a way as to enhance the acidity of the hydrogen atom, and in the present case a terminal carbonyl group, immediate difficulties arise. The only way in which such conditions can be satisfied is to adopt Fischer's (1944–5) suggestion of a condensation of glucose-6-phosphate in the following way:

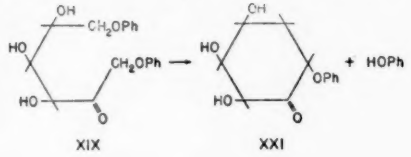


(40)

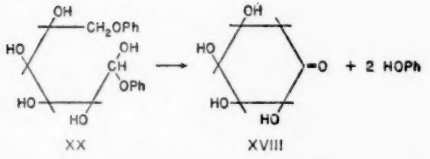
The resulting carbocycle is an inositol, and inositol might be formed in this way in plants; but in order to convert this inositol

into XVIII recourse must be had to a sequence of oxidation-reduction reactions acting at carbon atoms chosen arbitrarily so as to achieve the desired result.

By far the more satisfactory route is that involving the displacement of a phosphoryloxy group. Robinson (1927) has suggested that such a displacement could account for the origin of certain sugars in nature. The necessary conditions for ring closure by such a reaction are the presence of a phosphoryloxy group and a suitably disposed, potentially anionic carbon atom. Two likely routes in which these conditions are fulfilled are those through (41) fructose-1,6-diphosphate (XIX) and (42) a hypothetical glucose-1,6-diphosphate (XX) (Geissman, 1949*a*):

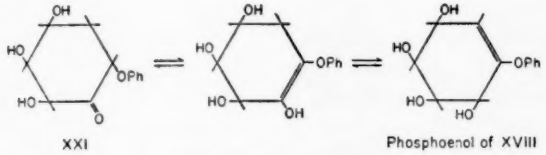


(41)



(42)

Scheme (42) leads directly to the desired intermediate XVIII. The product XXI from reaction 41 could be converted to XVIII by the changes



(43)

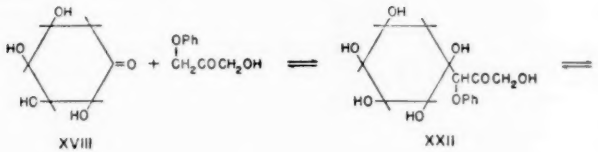
but this isomerization is, again, an arbitrary one.

FORMATION OF $C_6(B)$-C_3. The triose from which the C_3-fragment is derived seems most likely to be either dihydroxyacetone phosphate (DAP) or phosphoglyceraldehyde (PGA), both of which play a central role in cellular metabolism. The addition of

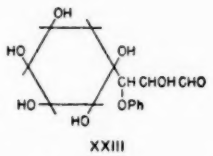
DAP to a carbonyl group by an aldol condensation is typified by the focal reaction in glycolysis:

$$\text{DAP} + \text{PGA} \rightleftharpoons \text{fructose-1,6-diphosphate.} \qquad (44)$$

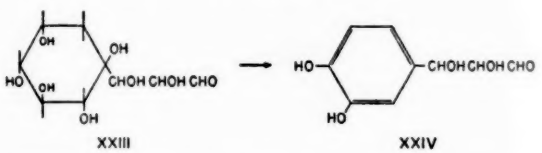
Aldol condensation of DAP with the precursor XVIII would be formulated as follows:



(45)



Dehydration of XXIII with the removal of three molecules of water leads to what may be regarded as the prototype XXIV of the common 3,4-dihydroxy C_6(B)-C_3-unit.



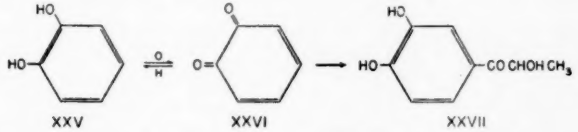
(46)

The direction of the dehydration at the 4,5-positions to eliminate the 5-hydroxyl group rather than the 4- may be related to the unique character of the 4-hydroxyl group: its freedom from glycosylation in the anthocyanins (where it is the 4′-hydroxyl), its freedom from methylation in the majority of (but not all) other cases (as in coniferyl alcohol (3-OCH_3-4-OH), syringyl alcohol (3,5-diOCH_3-4-OH), etc.). These observations suggest that the 4-OH in XXIII may not be free, but may be joined to some "protecting" group during the early stages of formation of the finally aromatized ring (cf. Seshadri, 1948). It is suggestive that the 4-hydroxyl group of glucose is the position most frequently involved in the formation of polysaccharide bonds.

The precursor XXIII can, by dehydrogenation instead of dehydration processes, give rise to C_6(B)-units as highly hydroxy-

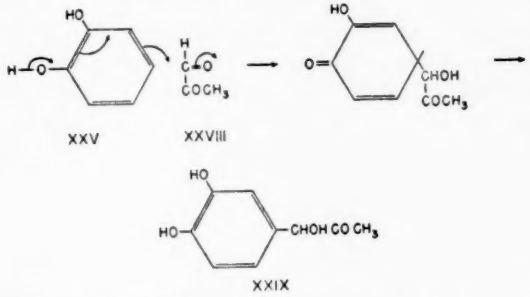
lated as 2,3,4,5-tetrahydroxy, as in apiole, 2,5-dimethoxy-3,4-methylenedioxy-1-allylbenzene, and several similar constituents of the essential oils of certain Umbelliferae.

An alternative theory of the formation of $C_6(B)$-C_3 is found in Hibbert's discussion of the lignin problem (1941). He has suggested that the C_6-precursor is (the already aromatized) catechol XXV, which is in oxidation-reduction equilibrium with the corresponding *o*-quinone XXVI, and that the C_6-C_3-prototype XXVII is formed as follows:



XXV XXVI XXVII (47)

The nature of the C_3-fragment and the kind of reaction responsible for its union with the catechol nucleus are not detailed by Hibbert, but a plausible reaction, involving methylgyloxal (XXVIII) and catechol (XXV), may be written as follows:



XXV XXVIII (48)

XXIX

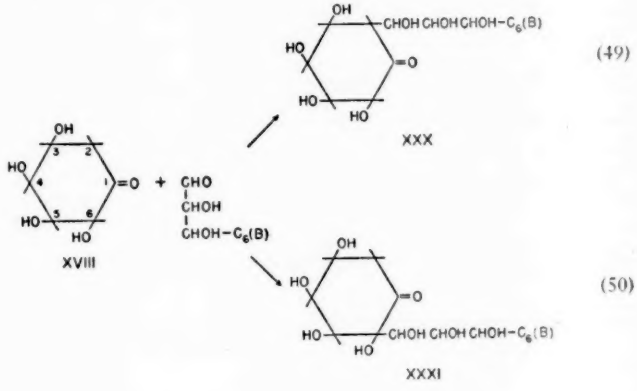
This reaction leads to a product (XXIX) in the same oxidation state as that written by Hibbert.

Rao and Seshadri (1943) have suggested the attachment of $C_6(B)$-C_3 to $C_6(A)$ in a similar manner (see below).

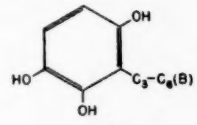
The occurrence in nature of methyl anthranilate and damascenin offers additional support for the suggestion that in C_6-C_1-compounds, the side chain is attached to the carbon atom which corresponds to the number one carbon atom of the original hexose. All of the naturally-occurring amino sugars are 2-aminohexoses. The cyclization of glucosamine by scheme 42 (p. 191) would lead

to a substance having the amino group in the position ortho to the side-chain. It is also suggestive that cyclization by way of a ketohexose form (scheme 41, p. 191) would not be a possible route for an amino sugar. It must be emphasized, however, that because of the limited distribution in plants of amino sugars and aniline derivatives (such as methyl anthranilate), their existence cannot be regarded as constituting compelling evidence in questions concerning the biosyntheses of non-nitrogenous compounds, since they may owe their occurrence to highly specific and unique processes. The parallels mentioned are noteworthy, however.

THE C_6(A)-FRAGMENT. The invariable presence of a hydroxyl group ortho to the side-chain; the possibility of the existence of a completely (penta) hydroxylated C_6(A)-nucleus; and the frequent occurrence of the phloroglucinol pattern of hydroxylation in the C_6(A)-part of the flavonoid compounds are in marked contrast to the nature of the C_6(B)-nucleus. The suggestion is therefore strong that either the C_6(A)-antecedent, to which condensation of C_6(B)-C_3 takes place, is not XVIII, or that the condensation, if on XVIII, takes place in a manner different from that which has been proposed for the generation of C_6(B)-C_3. The second of these alternatives appears the less satisfactory of the two. If XVIII is the precursor common to both C_6(B) and C_6(A), an aldol condensation between XVIII and C_6(B)-C_3 must then be regarded as involving a carbonyl group in the C_3-position and a —CH— or —CH_2— grouping in XVIII:



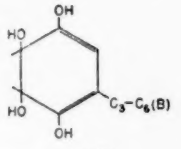
If XXX is the immediate product, it may be expected to lose two water molecules to give XXXII:



XXXII

The retention of the 4-hydroxyl group and the loss of the 5- in the transformation of XXX to XXXII is in conformance with the course suggested for the aromatization process leading to C_6(B) as described earlier. Clearly XXXII does not possess the typical C_6(A) hydroxylation pattern; indeed, the pattern of XXXII does not occur in any known flavonoid compounds from natural sources.

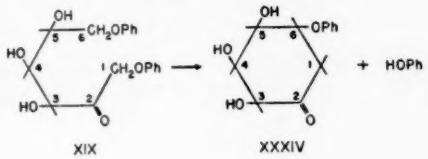
Starting from XXXI, dehydration with the retention of 4-OH, and enolization of the carbonyl group at C_1, leads at first to XXXIII:



XXXIII

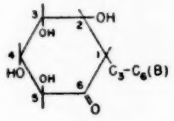
The retention of 4-OH requires the loss of 5-OH; and since this leads to a product having but one hydroxyl group ortho to the side-chain, the most ubiquitous feature of the C_6(A)-unit is lacking. Thus it appears that this route lacks the high degree of plausibility that must be sought in speculative reconstruction of this kind.

If the antecedent of C_6(A) is not XVIII, but another hexose-derived cyclitol, the application of the same requirements which have so far been observed makes possible the selection of a probable alternative. The cyclization of fructose-1,6-diphosphate XIX by a mechanism reminiscent of that proposed by Fischer for inositol formation may be written as follows:



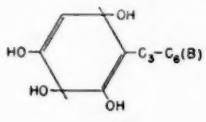
(51)

The aldol condensation between C_6(B)-C_3 and the —CH_2— group of XXXIV leads to XXXV (in which the configuration of the product except for carbon atoms 1 and 2 is shown):



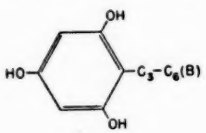
XXXV

Dehydration (with loss of 3-OH) and enolization lead to XXXVI.



XXXVI

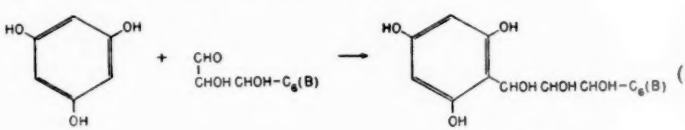
If the next dehydration occurs in such a way as to retain the hydroxyl group ortho to C_3-C_6(B), the product is XXXVII.



XXXVII

This has the ubiquitous and typical C_6(A) phloroglucinol hydroxylation pattern.

Rao and Seshadri (1943) have formulated the C_6(B)-C_3 to C_6(A) condensation as follows (e.g., 52):



(52)

XXXVII

The result of this condensation is the same as that arrived at above (XXXVII). There is ample reason to regard this route as possible in the cell under physiological conditions, since the anionoid reactivity of phloroglucinol is high, and such a condensation, from the mechanistic viewpoint, simulates closely the aldol

condensation. The chief disadvantage of this scheme is that the parent phenols, such as phloroglucinol in the example, occur rarely or not at all in the plants which contain the flavonoid compounds derived from them. This objection could be answered by supposing that the phenols in question are consumed largely or wholly in the formation of C_6-C_3-C_6-compounds.

THE OXIDATION LEVELS OF C_3. Examples of C_6-C_3-compounds with various degrees of oxidation of the C_3-chain have been tabulated in an earlier section. According to the Robinson-Seshadri hypothesis of a C_6(B)—CHOH—CHOH—CHO precursor, the derivation of substances as diverse as C_6—$CH_2CH{=}CH_2$, C_6—CH=CH—COOH, and the unknown C_6—$COCH_2COOH$ (whose transient existence may be inferred from the existence of C_6—$COCH_3$), must be the result of reductive and oxidative changes in the C_3-fragment. A scheme to indicate the course of such changes has been proposed by Hibbert who has discussed the relationship of the C_6-C_3-units, typified by coniferyl alcohol and syringyl alcohol, to lignin formation and to plant syntheses of other kinds. Hibbert has drawn an analogy between the oxidation-reduction changes which can occur in the C_3-side-chain and those proposed by Szent-Györgyi (1934) to explain the role of C_4-dicarboxylic acids in animal respiration. Although Szent-Györgyi's proposals have undergone considerable modification in the years since their original publication, the transformations occurring in the C_3-side-chain in Hibbert's scheme bear a sufficiently close relationship to many of the changes now known to occur in the respiratory metabolism of plant and animal tissues to warrant their consideration at the present time. As has been pointed out in an earlier section of this review, the main features of the enzymatic breakdown of carbohydrates and the transformations of their breakdown products in animal tissues have been demonstrated in plants as well. It is important to emphasize that there is no need to suppose the existence in plants of a unique system, peculiar to plant tissues, to account for the larger part of the respiration. Moreover, there is no need to preclude the suggestion that still-unrecognized chemical transformations similar in kind to those recognized in the tricarboxylic acid (Krebs-Hensleit) cycle may occur in chemically-similar systems. Hibbert's theory (1941) is illustrated by the following chart (Chart 1):

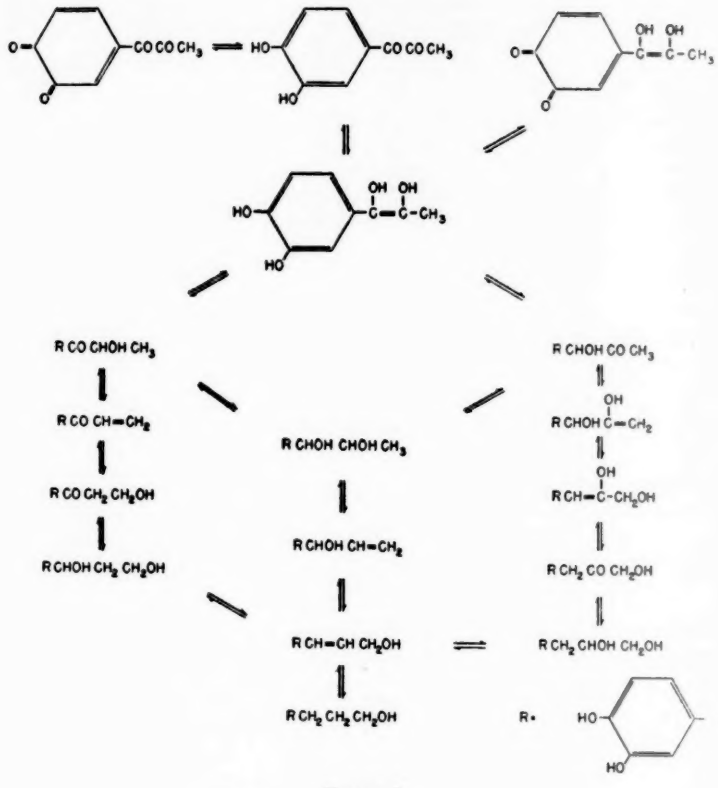
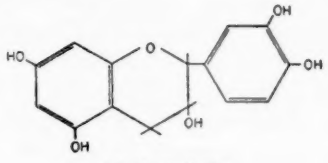


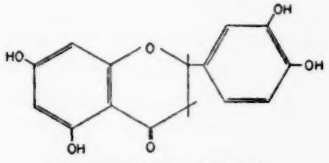
CHART 1.

In this scheme the addition and removal of hydrogen or water or the rearrangement of allylic systems represent the reversible changes shown. Although the theory includes the proposition that these changes constitute a complete metabolic (respiratory) network of the type illustrated, this aspect of it may now be minimized and attention directed to the possibility that such transformations represent a means by which a given plant may selectively produce a given C_6-C_3-compound from some precursor which may be characteristic of plants in general. This would mean that all of the enzymes represented by each of the steps shown exist in the plant kingdom, but that any one plant, species or genus may possess only a limited number of them.

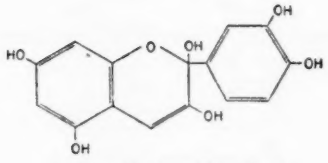
THE OXIDATION LEVELS OF C_3 IN C_6-C_3-C_6-COMPOUNDS. The range of oxidation of the C_3-portions of compounds having the C_6-C_3-C_6- (flavonoid) skeleton is illustrated by the following naturally-occurring compounds (the ubiquitous 3′,4′,5,7-tetrahydroxy pattern in the A- and B-rings is used in the examples selected):



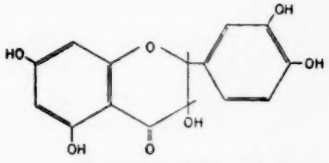
catechin ($C_{15}H_{14}O_6$)



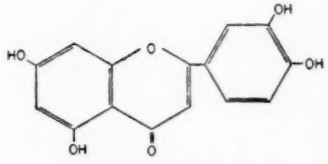
flavanone (eriodictyol, $C_{15}H_{12}O_6$)



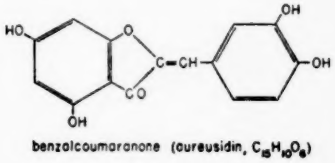
anthocyanidin (pseudobase form)
(cyanidin, $C_{15}H_{12}O_7$)



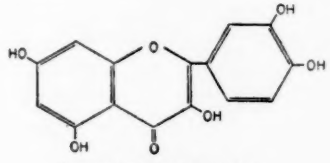
flavanonol (taxifolin, $C_{15}H_{12}O_7$)



flavone (luteolin, $C_{15}H_{10}O_6$)



benzalcoumaranone (oureusidin, $C_{15}H_{10}O_6$)

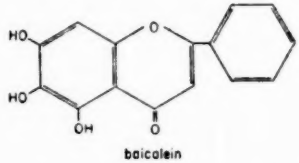


flavonol (quercetin, $C_{15}H_{10}O_7$)

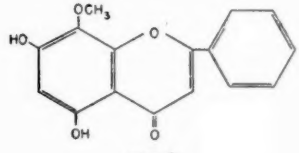
There are several lines of evidence which suggest that the oxidation level (i.e., the hydroxylation pattern) of the A- and B-rings is established prior to the oxidation (or reduction) of the C_3-fragment to its final state. The interconversion, for example, of a flavonol to an anthocyanidin is a theory which has been dis-

cussed earlier in this review and which includes the added supposition that the oxidation level of C_3 is fixed not only after the aromatization of A and B, but also after the closure of the heterocyclic ring. It has been pointed out that evidence for the latter suggestion is not convincing. There are, however, many examples of the co-occurrence of pairs of substances having the same pattern of hydroxylation in the A- and B-rings, but different levels of C_3-oxidation. *Rhus succedanea* Kudo contains fustin (254) and fisetin (211) (Oyamada, 1934); *Ampelopsis meliaefolia* Kudo contains the flavanonol * ampelopsin (256) and myricetin (231) (Kotake and Kubota, 1946); *Prunus serotina* and *Nothofagus Dombeyi* Blume contain Katsuranin (253) and naringenin (240) (Pew, 1948); *Eriodictyon californicum* contains homoeriodictyol (247) and chrysoeriol (196) (Tutin and Clewer, 1909); *Cosmos sulphureus* contains luteolin (194) and quercetin (217) (Geissman, 1942); and hesperetin (248) and diosmetin (197*a*) are found in many members of the Rutaceae (Klein, 1932).

The occurrence in *Scutellaria baicalensis* of baicalein and wogonin

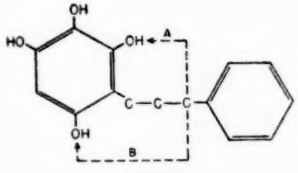


baicalein



wogonin

has been regarded (Rao and Seshadri, 1943) as evidence that this plant produces a precursor



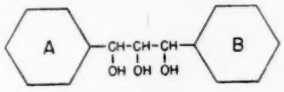
XXXVIII

which by closure of the heterocyclic ring by route A leads to wogonin, and by route B to baicalein.

* An anthocyanin isolated from *Ampelopsis quinquefolia* and thought to be identical with oenin and cyclamin is also called ampelopsin (274*a*). The hydroxylation pattern of this compound is like that of ampelopsin (the flavanonol) and myricetin except that the 3'- and 5'-hydroxyl groups of the latter compounds are methylated in the anthocyanin.

The presence of the 3′-methoxyl group in homoeriodictyol and chrysoeriol, and of the 4′-methoxyl group in hesperetin and diosmetin provide further evidence for the view that the 2-aryl residue is completely elaborated before the nature of the C_3-fragment is established. It would appear improbable that the same gene (or enzyme) would be capable of bringing about the methylation of one given position in both a flavanone and flavone, or in two compounds differing in the nature of the C_3-portion of the molecule; or that two genes exist in each of the plants, each of which has the ability to methylate the same position in two different substances. The simplest view is that a single substance is methylated through the action of a single gene, and the resulting product is then modified along divergent paths to the final flavone and flavanone.

The modification of the C_3-portion of the Robinson-Seshadri precursor XXXIX (cf. XII):



XXXIX

could proceed in the following ways (Chart 2):

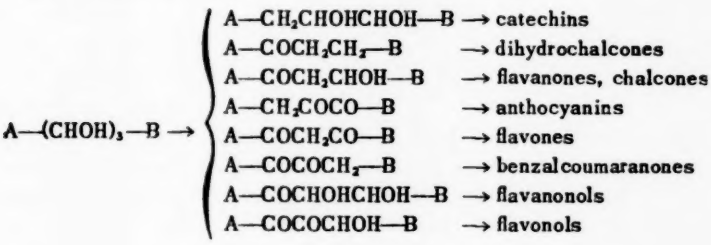
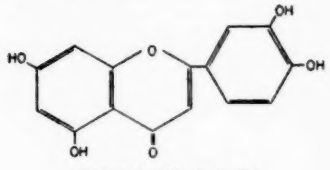
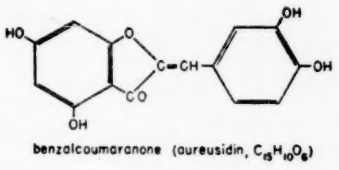


CHART 2.

The occurrence in yellow blossoms of *Antirrhinum majus* of luteolin and aureusidin

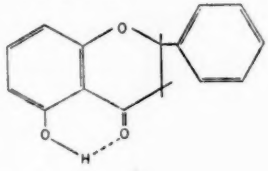


flavone (luteolin, $C_{15}H_{10}O_6$)



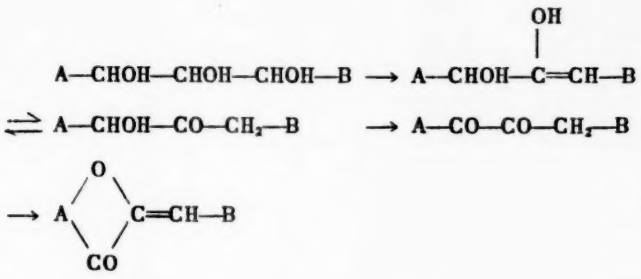
benzalcoumaranone (aureusidin, $C_{15}H_{10}O_6$)

represents additional evidence for the hypothesis under consideration. These compounds possess the same hydroxylation pattern in the A- and B-rings and the same oxidation level in C_3, but they are not interconvertible by any direct or simple process. Their derivation from the original A—CHOH—CHOH—CHOH—B can be formulated in more than one way, depending upon the sequence of steps assumed. There is no sound basis for selecting one of the possible sequences over others, but certain of the chemical properties of flavone derivatives possessing both a 4-carbonyl group and a 5-hydroxyl group make it possible to select what appear to be the more probable routes. The naturally-occurring chalcones are for the most part resorcinol derivatives (in the A-ring). When the phloroglucinol pattern of hydroxylation is present, chalcones do not appear to exist as such in the cell, the isomeric flavones being present. The change chalcone $\rightleftharpoons$ flavanone is a reversible acid-base catalyzed reaction which is strongly on the side of the flavanone when a 5-hydroxyl group is present. This is undoubtedly due to the stabilizing effect of hydrogen bonding of the following kind (XL):



XL

Similar stabilization is possible in flavones, flavonols, etc. It may be inferred from these observations that in the modification of A—CHOH—CHOH—CHOH—B into the benzalcoumaranone precursor A—CO—CO—CH_2—B, no intermediate stage having the partial structure A—CO—C(OH)—B **is involved, since if A** contained the phloroglucinol pattern of hydroxylation, such an intermediate would probably be stabilized by undergoing ring closure. The course of benzalcoumaranone synthesis may therefore be as follows:



(53)

A question which arises is whether the carbonyl group adjacent to the A-ring is produced at an early stage in the modification of C_3, or at a later stage. Purely on the basis of the arguments presented in the preceding paragraphs it may be suggested that the carbonyl group is elaborated near the end of the process, since otherwise the process would be interrupted by ring closure when the stage A—CO—C—C—B (OH) was reached.

The modification of C_3 according to the above scheme may be assumed to occur in a manner similar to that postulated by Hibbert; that is, simple processes of dehydration and dehydrogenation are probably involved. The following chart shows the reactions which could lead to the A-C_3-B-structures shown on p. 201; i.e., the immediate precursors which by ring closure lead to the various flavonoid substances (Chart 3).

It is noteworthy that in at least two cases (fustin and fisetin; ampelopsin and myricetin), a flavanonol and a flavonol with identical hydroxylation patterns in the A- and B-rings (neither pattern being the ubiquitous 3,3′,4′,5,7) occur together in the same plant. In the scheme above flavanonols and flavonols are derived from the common precursor through the same initial stage.

EVIDENCE FROM THE STRUCTURES OF THE ISOFLAVONES. The naturally-occurring isoflavones possess structures which, aside from the point of attachment of the aryl residue to the chromone ring, have many features in common with the flavane derivatives:

a) The B-rings are in most cases 3- or 3,4-hydroxylated.

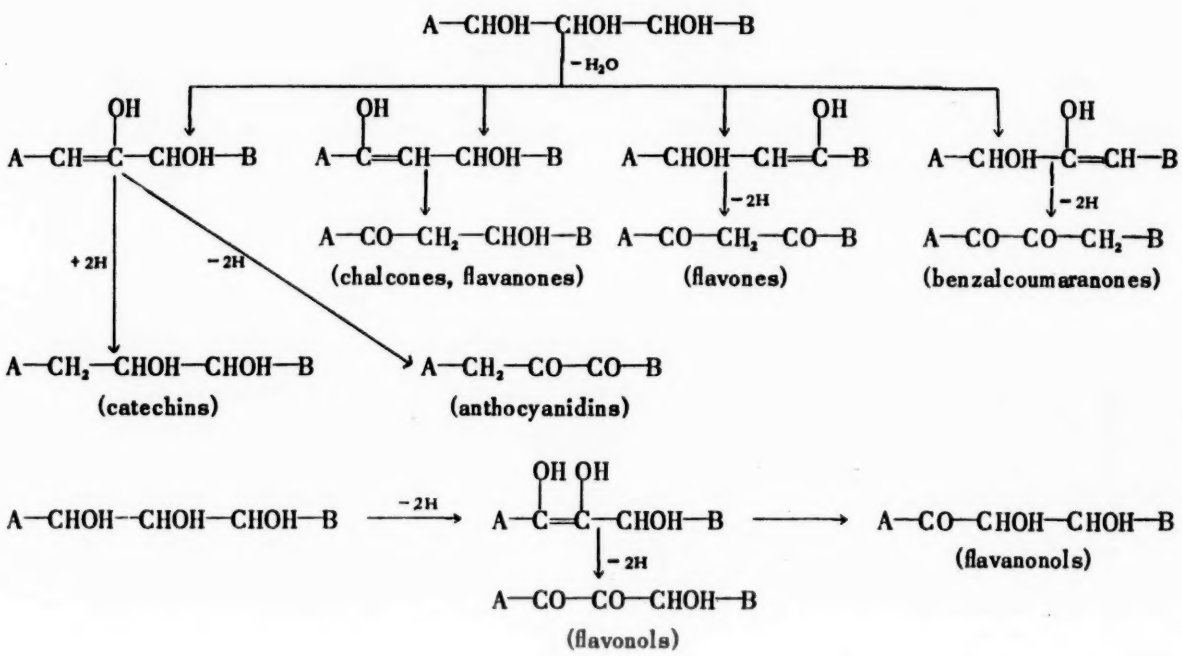
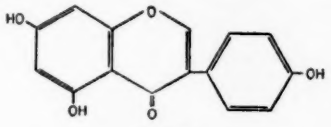

A—CHOH—CHOH—CHOH—B
−H2O
A—CH=C—CHOH—B
OH
A—C=CH—CHOH—B
OH
A—CHOH—CH=C—B
OH
A—CHOH—C=CH—B
OH
+2H
−2H
−2H
−2H
A—CO—CH2—CHOH—B
(chalcones, flavanones)
A—CO—CH2—CO—B
(flavones)
A—CO—CO—CH2—B
(benzalcoumaranones)
A—CH2—CHOH—CHOH—B
(catechins)
A—CH2—CO—CO—B
(anthocyanidins)
A—CHOH—CHOH—CHOH—B
−2H
OH OH
A—C=C—CHOH—B
−2H
A—CO—CO—CHOH—B
(flavonols)
A—CO—CHOH—CHOH—B
(flavanonols)

CHART 3.

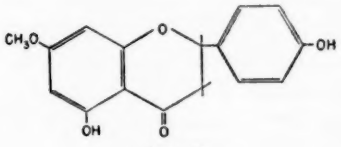
b) The A-rings possess the resorcinol, phloroglucinol or more complex patterns of hydroxylation, as do the A-rings of flavane derivatives.

One of the most widely-distributed hydroxylation patterns found in the isoflavones is that found in genistein (283*a*) and its monomethyl ethers, prunetin (286) and biochanin (285)

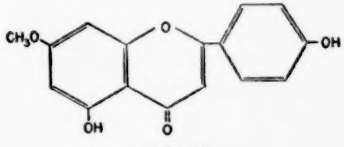


genistein

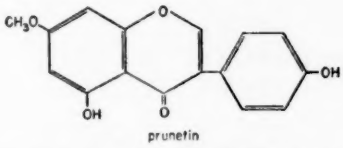
It is noteworthy that *Prunus puddum* (bark) contains sakuranetin, genkwanin and prunetin:



sakuranetin

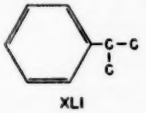


genkwanin



prunetin

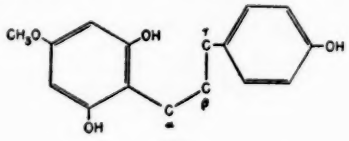
All of these are 7-methyl ethers of 5,7,4′-trihydroxycompounds. The co-occurrence of the similarly substituted flavanone and flavone is accountable for on the grounds of theories already discussed, but the extension of any of these hypotheses to cover the biosynthesis of the isoflavones presents obvious difficulties. The C_6(B)-C_3-unit of the isoflavone has the basal structure XLI.



XLI

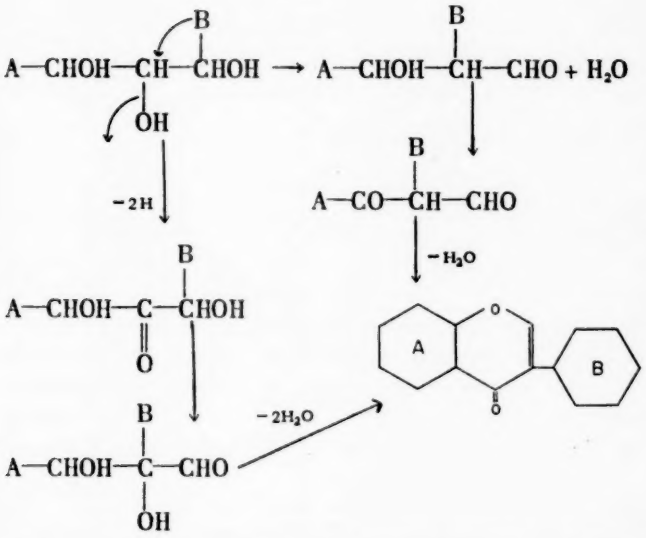
Formation of the precursor of this unit by condensation of a hexose-derived cyclitol with the second carbon atom of a triose

(instead of the first, as has been suggested in the case of the C_6-C_3-compounds and the flavane derivatives) is an obvious suggestion but one whose chief recommendation lies in its facility. Except in the terpenoid compounds and in tropic acid, the existence of C_6-C_3-compounds possessing side-chains derived from the isopropyl residue has not been reported. Since the C_3-C_6(B)-portion of flavane derivatives almost certainly has a biogenetic correspondence to the widely-occurring C_6-C_3-compounds of the coniferyl alcohol, eugenol, etc., types, it might be expected that if isoflavones were derived in a similar manner by way of a condensation of a C_6(A)-unit with a C_6(B)-iso-C_3-precursor, the latter unit would survive in some plants and be recognized as a discrete C_9-compound. Since this is not the case, it is permissible to seek the possible origin of the C_6(B)-iso-C_3-fragment from a C_6(B)-C-C-C-fragment. From the argument given above, it would also be reasonable to infer that if C_6(B)-iso-C_3 arises from C_6(B)-*n*-C_3, the transformation is most likely to occur at the C_6(A)-C_3-C_6(B)-stage. This inference is supported by the example of *Prunus puddum*, in which the two flavane derivatives and the isoflavone have not only the same hydroxylation pattern but the same position of methylation as well. The simplest assumption, and one which has formed the basis of the hypothesis elaborated earlier in this review, is that the precursor common to all three of these substances has the partial structure XLII.



XLII

The derivation of the isoflavone must then be the result of a rearrangement of the γ-aryl group to the β-position. Although this suggestion is highly speculative, the nature of the C_3-fragment of the common antecedent is uniquely constituted to undergo a rearrangement of the pinacol or Wagner-Meerwein (i.e., 1,2-) type. The following formulations are given only as illustrations of routes which are mechanistically feasible, since there exists no sound basis upon which to sustain a more explicit proposal:

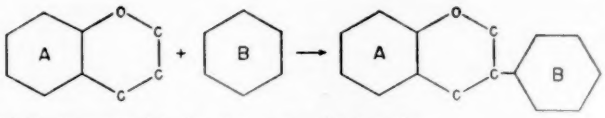


(54)

It is, of course, characteristic of rearrangements of this type that the point of attachment of the migrating aryl group is the same in the initial and final products; that is, if B in A—C—C—C—B is *p*-hydroxyphenyl, B in the rearrangement product A—C—C—C is also *p*-hydroxyphenyl.

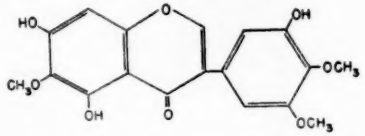
It cannot be assumed, however, that because biosynthesis of isoflavones by the rearrangement of the common precursor is mechanistically acceptable and bears a self-consistent relationship to the genesis of flavane derivatives discussed above, the hypothesis is the only one that need be considered. But to devise another, and at the same time adhere to the fundamental precepts of biochemical analogy and mechanistic validity, would require the erection of a hypothesis having fundamental features quite distinct from those which have formed the basis of most existing interpretations of the biosynthesis of flavane derivatives. Some of the objections to certain otherwise plausible schemes may be suggested:

a) The condensation of a C_6(A)-C_3-fragment with a preformed C_6(B)



(55)

would be improbable in the case of irigenin,



since the most probable position of substitution in 2,3-dimethoxyphenol (acting as C_6(B)) would be 4- rather than 5-.

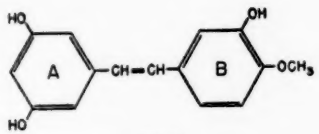
b) The condensation of C_6(B)-iso-C_3 with C_6(A) is mechanistically and biochemically plausible but, as was pointed out above, lacks the support which would be furnished by the existence of C_6(B)-iso-C_3-compounds in nature.

c) The simultaneous combination of C_6(A), C_3 and C_6(B) is unlikely on the ground of the improbability that such combinations of three molecules, suitably oriented, would occur at a reasonable rate, even under the influence of a specific enzyme.

STILBENES AND XANTHONES. The naturally-occurring stilbenes and xanthones are classes of compounds which, although possessing the basal structures of C_6-C_2-C_6 and C_6-C_1-C_6, respectively, appear to be related to the flavonoid substances. Both stilbenes and xanthones contain aromatic nuclei which are hydroxylated according to the general pattern of the common C_6-C_3-C_6-compounds; and it is convenient to regard them as being composed of flavonoid-like A- and B-rings, joined by one or two carbon atoms.

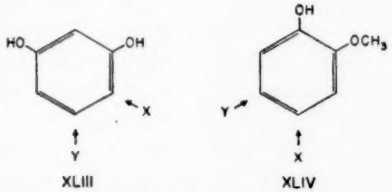
The naturally-occurring stilbenes (Table 20) are characterized by the presence of a ring (B) which is substituted in the manner characteristic of the C_6(B)-ring of the flavonoid compounds. There are known the phenyl (pinosylvin (308)), *p*-hydroxyphenyl (resveratrol (311), pterostilbene (312)), 3-hydroxy-4-methoxyphenyl (rhapontigenin (314)) and 2,4-dihydroxyphenyl (hydroxyresveratrole (313), chlorophorin (315)) rings, which have their counterparts in the B-rings of galangin (203), kaempferol (206), hesperidin (248) and morin (222), respectively.

The other aryl residue of the stilbenes possesses a hydroxylation pattern which is unique in that it is a 3,5-dihydroxyphenyl residue. The structure of rhapontigenin is shown below to illustrate these features:



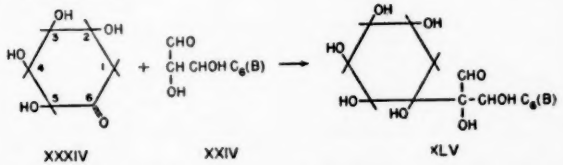
A-C_2-B
rhapontigenin

It may at once be concluded that the attachment of the C_2-fragment does not occur by a substitution into the preformed (aromatic) A- and B-residues, since resorcinol XLIII and guaiacol XLIV (the A- and B-rings, respectively, of rhapontigenin) preferably undergo substitution in the positions shown as X below, while substitutions at Y would have to occur to lead to rhapontigenin by such a route.



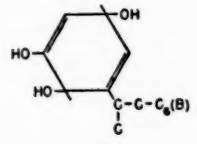
XLIII XLIV

If, however, the precursor of the C_6(B)-C_2-portion of the molecule is again a typical Robinson-Seshadri C_6-C_3-unit XXIV, the step leading to the stilbenes may then be a condensation of the second (β) carbon atom of this precursor with a C_6(A)-precursor. Since the genesis of C_6(A)-C_3-C_6(B) has been represented in the foregoing discussion as proceeding by way of an aldol condensation involving an anionoid carbon atom in the C_6(A)-precursor and a carbonyl group in C_3, it is a reasonable inference that when the second carbon atom in C_3 is involved, the condensation with the C_6(A)-precursor XXXIV takes place between a carbonyl group in the latter and an anionoid carbon atom in the C_3-fragment:



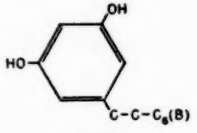
XXXIV XXIV XLV (56)

If the dehydration of the carbocyclic ring of XLV again proceeds with retention of 4-OH, the loss of two molecules of water leads to XLVI.



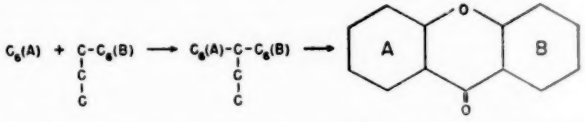
XLVI

The subsequent loss of the third molecule of water with the removal of 3-OH, followed by the degradation of the branched three-carbon fragment with the retention of the carbon atoms joining the rings, leads to XLVII:



XLVII

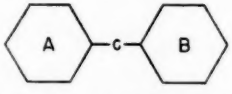
The xanthones are too limited in distribution to enable a "characteristic" pattern to be discerned. It is an attractive supposition that they are derived from a precursor formed by the condensation of a C_6(A)-antecedent with the carbon atom of C_6(B)-C_3 which is adjacent to the C_6(B)-ring, followed by the loss of two carbon atoms, as shown in equation 57:



(57)

There is, however, insufficient support from structural evidence to sustain this suggestion.

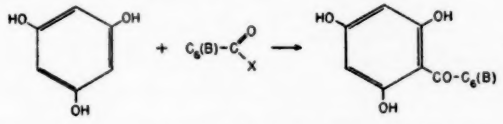
BENZOPHENONES. The naturally-occurring benzophenones are closely related to the flavonoid substances, according to the criteria used in this review. They may be represented as C_6(A)-C-C_6(B)-compounds of the following type:



in which the A- and B-rings are hydroxylated in the patterns characteristic of the corresponding rings of the C_6-C_3-C_6-substances. The common naturally-occurring benzophenones of cotobark, and maclurin all contain a phloroglucinol-derived A-ring,

and B-ring with the typical C_6(B) hydroxylation patterns referred to repeatedly in the foregoing sections.

These observations afford no compelling evidence to support any one of several plausible biogenetic routes to these compounds. One of these (58) involves the condensation of phloroglucinol with a C_6(B)-carboxylic acid (or acid derivative):



(58)

An alternative synthesis related to this, biochemically plausible and resembling the combinations of C_6(A) and C_6(B)-C_3 discussed earlier, is as follows (59):

$$C_6(A)\ [e.g.,\ XXXIV] + C_6(B)\text{—}CO\ COOH \text{ or } C_6(B)\text{—}CHO$$

$$\longrightarrow C_6(A)\text{—}\underset{\displaystyle COOH}{\overset{\displaystyle OH}{\underset{|}{\overset{|}{C}}}}\text{—}C_6(B) \text{ or } C_6(A)\text{—}\overset{\displaystyle OH}{\overset{|}{CH}}\text{—}C_6(B) \qquad (59)^*$$

$$\longrightarrow C_6(A)\text{—}CO\text{—}C_6(B)\ [\text{or} + CO_2].$$

The relationship of this route to that earlier proposed for flavonoid compounds is a close one, and the application of the principles observed in the latter scheme would offer a means of arriving at a phloroglucinol-type A ring.

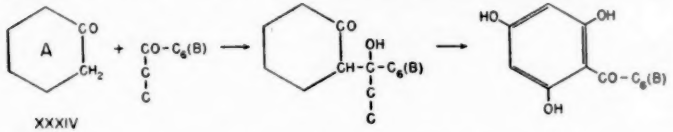
Another route is through the condensation of a C_6(A)-fragment with the α-carbon atom of a C_6(B)-C_3-moiety, followed by the loss by degradation reactions of the two lateral carbon atoms:

$$C_6(A) + \underset{\displaystyle C}{\overset{\displaystyle C\text{—}C_6(B)}{\underset{|}{\overset{|}{C}}}} \rightarrow C_6(A)\text{—}\underset{\displaystyle C}{\overset{\displaystyle C\text{—}C_6(B)}{\underset{|}{\overset{|}{C}}}} \rightarrow C_6(A)\text{—}CO\text{—}C_6(B). \qquad (60)$$

If the latter course represents the actual biogenetic origin of the benzophenones in plants, the postulates of an earlier section (p.

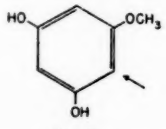
* In this scheme (59), C_6(B) is already aromatized, while C_6(A) is the non-aromatic precursor.

192) further require that, in order to arrive at a phloroglucinol pattern in A, the condensation take place between a carbonyl group in $C_6(B)$-C_3 and an anionoid methylene group in $C_6(A)$:



XXXIV (61)

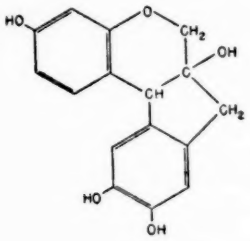
Of the benzophenones occurring in coto-bark, at least one bears a methyl group in such a position (4-) as to suggest that the methylation followed, rather than preceded, the condensation of the $C_6(B)$-C-fragment to $C_6(A)$, if scheme 58 represents the biosynthetic process. This conclusion is based upon the probability that phloroglucinol monomethyl ether (XLVIII) would be expected to condense in the position ortho to the methoxyl group:



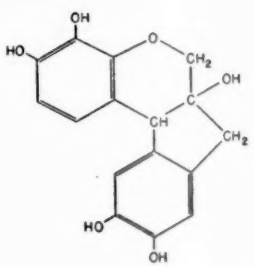
XLVIII

The existence of methylhydrocotoin (2,4,6-trimethoxybenzophenone) supports the suggestion that methylation occurs late in the synthetic process, since the anionoid character of phloroglucinol trimethyl ether is so low as to make improbable its participation in a condensation reaction under the conditions existing in the living cell.

BRAZILIN AND HEMATOXYLIN. Brazilin XLIX and hematoxylin L possess structures which appear, from their hydroxylation patterns, to be related to the flavonoid substances.

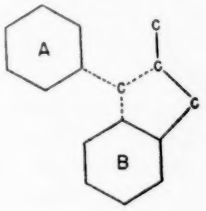


XLIX
brazilin



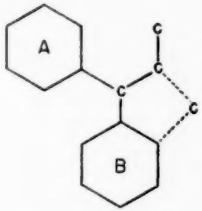
L
hematoxylin

These may be regarded as examples of C_6(A)-C_3-C_6(B)-compounds in which the added carbon atom constitutes a bridge between the A- and B-rings and the C_3-chain (LI):



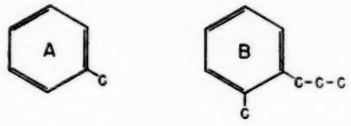
LI

or in which the added carbon atom forms a bridge between C_6(B)- and the C_3-chain (LII)



LII

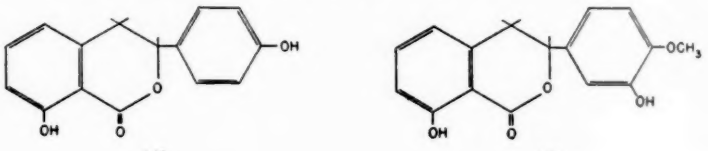
Since the B-rings of both brazilin and hematoxylin contain the dihydroxy grouping (of catechol) rather than the hydroxymethoxy grouping (of guaicol), it is impossible to decide even provisionally which group of three carbon atoms represents the C_3-moiety of the C_6(B)-C_3-progenitor. Consequently the biosynthetic origin of these interesting compounds can be suggested only in the general way which has been represented above. Of the two precursors LI and LII, the latter is somewhat the more satisfactory, since the likelihood of the intervention of a carbon atom (from a "formaldehyde equivalent"—see below) to form another ring seems greater in the latter case LII than in LI, where the three components (A, C_3-B and C) must unite at once, or a precursor of one of the uncommon types, LIII or LIV,



LIII LIV

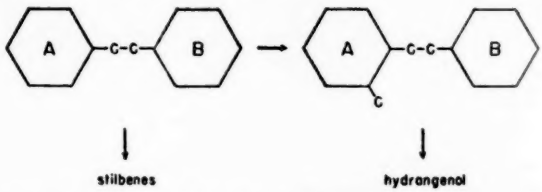
must be formed in a preliminary stage.

HYDRANGENOL. That the formation of brazilin and hematoxylin by way of a precursor such as LIII or LIV is not to be dismissed summarily is indicated by the occurrence of hydrangenol LV (and the related phyllodulcin LVI) in *Hydrangea opuloides.*



LV hydrangenol LVI phyllodulcin

The origin of these compounds by the introduction of the carbon atom found as the (lactonized) carboxyl group allows the inference that their biosynthesis proceeds along paths paralleling that of stilbenes, with the additional step in which a carbon atom is introduced into A at the stage of



(62)

If this reaction is considered possible, the formation of LIII by the introduction of a carbon atom into a C_6-unit must be regarded as no less possible.

The preceding discussion of brazilin, hematoxylin, hydrangenol and phyllodulcin must be regarded as even more tentative than the obviously speculative treatment of the biogenesis of the flavonoids and related compounds. This is so primarily because these four compounds form an isolated group of substances whose relationship to the flavonoids may reasonably be inferred, but concerning which the arguments advanced are not supported by informa-

tion concerning the structural features of a large number of related compounds.

ATTACHMENT OF SUGAR RESIDUES. Although there are no known examples of glycosylation on hydroxyl groups in the 2'- or 6-positions, the sugar residues found in the naturally-occurring flavonoid glycosides may be attached to any of the other positions at which hydroxyl groups exist, namely, at 3',4',3,5,7 or 8 (flavone numbering). There are, however, certain preferred positions (from a statistical point of view):

a) Flavones and chalcones are predominantly glycosylated at the positions which correspond to the positions numbered 3 and 7 in the flavone.

b) Anthocyanins are always glycosylated at the 3 and 5 positions (3- or 3,5-).

c) Flavonoids containing a 4-carbonyl and a 5-hydroxyl group are rarely glycosylated at the 5-hydroxyl group.

These observations suggest that glycosylation occurs at a late stage in the synthesis, probably after the complete elaboration of the structure of the flavonoid compound. This conclusion is further supported by several additional facts:

a) The fact that the 5-hydroxyl group in flavones is only rarely glycosylated is in accord with the reluctance of this hydroxyl group to undergo methylation or acetylation under conditions which permit the ready substitution of hydroxyl groups in other positions. This relative reactivity of the 5-hydroxyl group is attributable to its interaction through hydrogen bonding with the carbonyl group in the 4-position (see p. 202). If the glycosylation occurred prior to the closure of the heterocyclic ring, the deactivating influence of a carbonyl group in the same relative position in the C_3-chain (i.e., alpha to the C_6(A)-nucleus) would be less pronounced than in the flavonoid structure, and glycosylation might be expected to occur ortho to the side-chain. It should be emphasized, however, that although the presence of a sugar residue at the 5-position is rare, it is not unknown.

In the anthocyanins, which lack a carbonyl group at 4, sugar residues are commonly and characteristically present at 5. In these compounds there is no strong influence, such as the 4-carbonyl group exerts in flavones, to prevent the glycosylation of the 5-hydroxyl group.

b) Bate-Smith (1949) has shown, by the use of paper chromatography, that in the maturing flower of *Coreopsis* the petal content of free butein diminishes and that of a butein glycoside increases with time. This strongly suggests the direct conversion of aglucone into glucoside instead of a more complicated course involving a synthesis and later destruction of aglucone, accompanied by a concomitant synthesis of glucoside in some independent manner.

The nature of the cellular process whereby the glycosidic linkage is formed by the reaction of a sugar (or a derivative, such as a phosphorylated sugar) and a hydroxyl group is not known, but it seems safe to ascribe it to the agency of glycosidases, many of which are known in plants, and which have the ability to hydrolyze the glycosides found in the same plants. Since the reaction is an enzymatic one, it may be assumed that its reversibility in the living cell can lead to the combination of the fragments which, in the test-tube, are the products of the glycosidic fission.

Reichel and Schickle (1942) take the view that chalcone and flavanone glycosides are formed by condensations of the glycosides of hydroxyacetophenones and hydroxybenzaldehydes, rather than by glycosylation of chalcones derived from the corresponding sugar-free reactants. They showed that under "physiological conditions" (pH approx. 8, room temperature), condensations of the following kind took place in yields of the order of 20% when the reaction was allowed to proceed from 80 to 100 days:

a) resacetophenone-4-β-D-glucoside + benzaldehyde → flavanone-7-β-D-glucoside

b) *o*-hydroxyacetophenone + *p*-hydroxybenzaldehyde-β-D-glucoside → flavanone-4′-β-D-glucoside.

Reichel (1944) has also demonstrated that these reactions are catalyzed by amino acids (sarcosine, alanine, glutamic acid, etc.) in dilute solution at ordinary temperatures. Under comparable ("physiological") conditions, the corresponding aglucones (e.g., resacetophenone and benzaldehyde) did not undergo condensation.

The significance of these experiments with regard to biosynthetic processes is questionable. They support the possibility that chalcone and flavanone glycosides may originate by way of such condensations, but in view of the low yields, the extended reaction

periods and the alkaline conditions employed, the condensations appear to simulate more closely the conventional method of synthesis of chalcones and their glycosides than a cellular process.

ORIGIN OF THE METHOXY AND METHYLENEDIOXY GROUPS. The occurrence in natural sources of methyl ethers and methylenedioxy derivatives of compounds possessing hydroxylated aromatic nuclei is general. Indeed, nearly all of the naturally-occurring polyhydroxy C_6-C_3-C_6- and C_6-C_3-compounds which are known are represented also by mono- or polymethylated counterparts. The following are representative groups of compounds related in this way:

a) delphinidin (*272*), petunidin (*273*), malvidin (*274*), hirsutidin (*275*);

b) naringenin (240), sakuranetin (241), isosakuranetin (242);

c) quercetin (217), rhamnetin (220), rhamnazin (221), isorhamnetin (218).

It has been suggested in a previous section of this review that the methylation reaction takes place at the C_6-C_3-C_6 stage but prior to the closure of the heterocyclic ring. Of the mechanism of the actual methylation reaction, however, little is known, and little speculation has been offered regarding it. Browne and Phillips (1935) have offered a theory of the origin of ether linkages in nature, suggesting their formation by the degradation of carbohydrate fragments but without, however, dealing explicitly with the problem of the origin of aromatic methoxyl groups. Browne and Phillips reject the view, promulgated many years ago by Pictet (1905), that formaldehyde furnishes the carbon atom which forms the CH_3— and —OCH_2O— groups. The argument they adduce against Pictet's suggestion is that *Penicillium*, grown on a medium in which glucose is the only source of carbon, is able to produce methoxydihydroxytoluquinone (Birkinshaw and Raistrick, 1931). This argument seems to have little validity unless it were certain that the mold was unable to produce formaldehyde by breakdown of the sugar, and carries the implication that the formaldehyde assumed by Pictet is a product of photosynthesis. It is, however, scarcely necessary to erect arguments of this sort against the "formaldehyde theory" of the origin of methoxyl groups, since evidence in support of such a hypothesis is entirely lacking.

The question of the presence of formaldehyde in the photosynthesizing green plant has long been a controversial one. Klein and Werner (1926) offered evidence that formaldehyde is an intermediate product in carbon dioxide assimilation by plants and claimed to have demonstrated its presence in leaves. Subsequent work has led to contradictory findings, and the matter is still unsettled (Rabinowitch, 1935; Tottingham, 1937). The occurrence of formaldehyde in leaves has been reported by others, among them Schweitzer (1916) who showed that glycine can be converted into formaldehyde, carbon dioxide and ammonia by the action of the polyphenol oxidase of the potato, and also that *Papaver* leaves showed the presence of formaldehyde after irradiation with sunlight but not when kept in the dark. It should be noted, however, that in this work the identification of formaldehyde was not rigorous but depended upon a color test. On the basis of recent studies of photosynthesis the concept of the intermediary role of formaldehyde is giving way to new information on the mode of carbon dioxide assimilation in green plants, and the compelling necessity for adducing evidence in support of the older theories is fast disappearing.

The concept of the participation, if not of formaldehyde, of some " formaldehyde equivalent ", in biosynthetic processes is, however, difficult to discard. That formaldehyde itself cannot with certainty be demonstrated in living plant tissues is an objection which could be disposed of by the argument that because of its great reactivity it would be largely or completely utilized at once and thus be present in the tissues in minute amounts, if at all. That a " formaldehyde equivalent "—some source of a single carbon atom, with reactive capabilities similar to those of formaldehyde—is available in plant tissues is strongly suggested by a number of observations:

a) The wide occurrence in plant substances of methoxyl and methylenedioxy groups, and the absence of alkyl ethers of other kinds (except those which are clearly of isoprenoid nature).

b) The compelling plausibility of those steps in existing theories of alkaloid biogenesis in which the key reaction is a ring closure with the addition of a single carbon atom, the reaction simulating in a striking way in its structural requirements in vitro reactions in which formaldehyde takes part (for example, Anet, Hughes and Ritchie, 1950).

c) The wide occurrence of methyl alcohol in the form of such esters as methyl salicylate, methyl anthranilate, pectins and chlorophyll.

d) The occurrence of such compounds as brazilin and hematoxylin, the structures of which are flavonoid in type, but which contain one "bridging" carbon atom in excess of the C_6-C_3-C_6-portion.

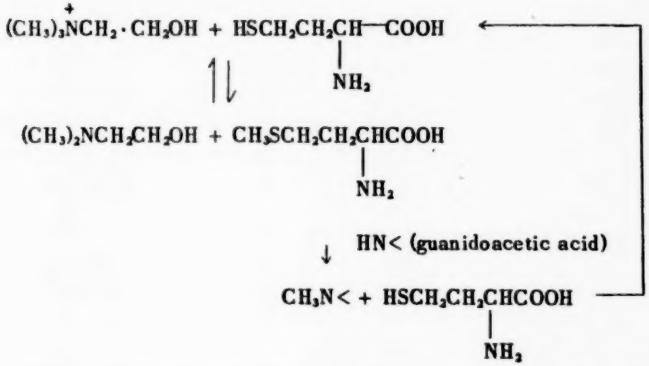
e) The common occurrence of the *N*-methyl group (and the fully methylated ammonium nitrogen of betaines) among alkaloids and related plant substances.

The possibility of the genesis of the *N*-methyl group by the reaction sequence (63)

$$>NH + [CH_2O]^* \longrightarrow >N{-}CH_2OH \longrightarrow >NCH_3 \qquad (63)$$

is supported by the ease with which a methylation of this kind is realizable in the laboratory.

The nature of biological methylations which occur in animal tissues and which involve the addition of a methyl group to a nitrogen atom has been elucidated by many recent studies, particularly those of du Vigneaud and his collaborators (du Vigneaud, 1948; Keller, Rachele and du Vigneaud, 1949). The process involved in certain of these reactions appears to bear little direct relation to those postulated in theories based upon the participation of formaldehyde, since the "transmethylation" reaction consists in the transfer of a methyl group as a unit. The process may be illustrated by the following:



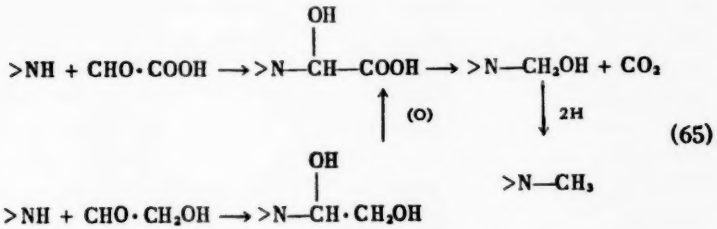
(64)

* The use of brackets signifies that a "formaldehyde equivalent" may be the actual species involved.

It is plausible to suppose that methylation by a similar mechanism is responsible for the formation in the plant of substances such as trigonelline, other betaines, and simpler *N*-methylated amines (Baldwin, 1947). However, Klein and Linser (1932), in an investigation of the in vivo synthesis of betaines (stachydrine and trigonelline), have shown that trigonelline synthesis is enhanced (over controls) to a greater degree by the injection of proline and urotropine (a formaldehyde source) than by proline alone. This suggests either the participation of formaldehyde in transmethylation in plants, or its utilization to form methyl groups in some other way. The transmethylation reaction has been demonstrated in wheat germ (Barrenscheen and Vályi-Nagi, 1942).

The results of the studies of biological methylation suggest that the animal organism is incapable of synthesizing methyl groups and can exist only if furnished a labile source of these units. Plants, on the other hand, do synthesize methyl groups. It is an obvious but too facile conclusion that, since the most conspicuous difference between green plants and animal organisms is the possession by the former of the photosynthetic mechanism, the source of the methyl groups in plants is ultimately to be found in the photosynthetic process. Although it has been emphasized earlier in this review that many of the simpler metabolic transformations are the same in living cells of all kinds, there is no reason to suppose that the ability of plants to methylate oxygen and nitrogen atoms may not be found in their possession of additional enzyme systems, outside of the photosynthetic mechanism itself, which are not found in animals and other heterotrophic organisms.

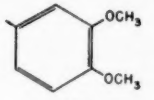
The question of what such systems may be is completely unsolved. The question of the chemical substances that may participate in the reactions they mediate is equally obscure, but Robinson has considered this problem and has suggested the possible identity of the "formaldehyde equivalents" (Robinson, 1917) with glyoxylic acid (Kolesnikov, 1948, 1949) or glycolic aldehyde. The conversion of these into methyl groups may be represented by the equations:



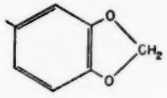
(65)

Bach (1939), in a study carried out before the transmethylation reaction had been recognized, suggested the methylation of guanidoacetic acid to creatinine by glyoxylic acid derived by the deamination of glycine; and Challenger et al. (1936, 1937, 1945) and Blackburn and Challenger (1938), who demonstrated the formation of methyl mercaptans from alkyl disulfides ($R_2S_2 \rightarrow RSH \rightarrow RSCH_3$) in mold cultures, have discussed the same possibility, adding the suggestion that formaldehyde itself may be formed by decarboxylation of the glyoxylic acid. However, Steensholt (1947) has shown that both glyoxylic acid and glycine are incapable of serving as methyl group sources in creatine formation in animal tissue (rabbit heart).

None of the recent theories and experimental findings regarding the origin of nitrogen-linked methyl groups have been applied to the problem of the origin of oxygen-linked methyl groups. It would appear premature to suggest that the methyl ethers in plants owe their origin to the transfer of methyl groups in toto from some methyl donor to oxygen, because of the close biogenetic relationship which appears to exist between the methoxyl group (LVII) and the methylenedioxy group (LVIII):



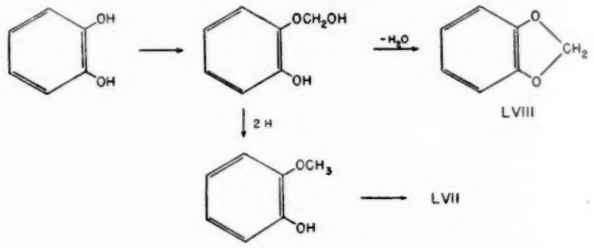
LVII



LVIII

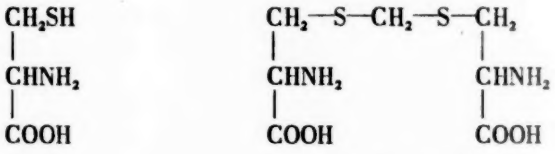
This relationship is most conspicuous among the plant alkaloids. Such substances as chelidonine and sanguinarine, anhalonine and anhalonidine, and cryptopine and protopine, which are botanically closely related, are characterized by the presence of the *o*-dimethoxy grouping in one and the methylenedioxy grouping in the other of each pair. It would seem highly unlikely that one of

these was produced through a methylation process involving the transfer of an intact methyl group and the other through a basically dissimilar process involving a cellular reagent possessing a —CHO or —OCH_2OH grouping. Thus it appears that there exists some process of biological methylation other than methyl transfer, and presumably one that proceeds through steps, the simplest representation of which is shown in equation 66:

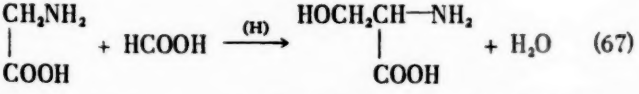


(66)

That some corresponding process can occur to lead to sulfur alkylation is indicated by the occurrence of djenkolic acid, a cysteine-derived amino acid found in the djenkol bean (*Pithecolobium lobatum*):



A substance which can play the role of a "formaldehyde equivalent" in animals is formic acid which has been shown (Sakami, 1948) to be the source of the added carbon atom in the conversion of glycine into serine in the rat (67):



(67)

Whatever the as yet unknown detailed process by which this reaction proceeds, it represents an overall reduction of formic acid and may be represented by the following formally equivalent steps

(68) (cf., Shemin, 1946; Ehrensvard, et al., 1947; Winnick, et al., 1948; Braunshtein and Vilenkina, 1949; Wood and Lorber, 1949):

$$\begin{aligned} &HCOOH \xrightarrow{2H} HCHO + H_2O \\ &HCHO + \underset{\underset{COOH}{|}}{CH_2NH_2} \longrightarrow \underset{\underset{COOH}{|}}{HOCH_2CHNH_2} \end{aligned} \qquad (68)$$

There is no basis for supposing that formic acid is or gives rise to the "formaldehyde equivalent" involved in the methylation and methylenedioxylation of oxygen and sulfur in plants and in the addition of a single carbon atom in the synthesis of alkaloids and other plant substances. However, it is worthy of note that while the presence of formaldehyde in plant tissues is inconclusive, formic acid has been recognized as being of wide occurrence (Bergmann, 1882), and the wide distribution of formic dehydrogenase in higher plants suggests the equally wide occurrence of its substrate (Davidson, 1949*a, b*). Numerous organisms—plant, animal, fungal and bacterial—possess enzymic mechanisms by means of which formic acid is formed and utilized (e.g., Chrzaszca and Zakomorny, 1933, 1935; Woods, 1936; Utter, et al., 1945; Strecker, et al., 1948).

COUMARINS. It has already been noted that among the simple coumarins—that is, those without furan rings—the hydroxylation pattern is ordinarily of the C_6(B)-ring type. Except for coumarin itself and melilotin (dihydrocoumarin), all of the naturally-occurring coumarins have a hydroxyl function in the 7-position (para to the C_3-side-chain), and many are hydroxylated in the 6- and/or 8-positions as well. On the other hand, hydroxylation in the 5-position (ortho to the side-chain), giving substitution of a type which appears to be related to the A-ring of C_6-C_3-C_6-compounds, occurs in only five simple coumarins. Of these, four (limettin (73), 7-methoxy-5-geranoxycoumarin (74), toddalolactone (75) and xanthoxyletin (103)) are derived from rutaceous plants, the former two from *Citrus limonum*. From the same species is also obtained the flavanone, citronetin (244), which has a methoxyl group in the usually-unsubstituted 2′-position. It is conceivable,

therefore, that ortho substitution in the B-ring, although generally quite rare, is a typical feature of some Rutaceae, and hence these 5-hydroxylated coumarins may actually be related to the B-ring also. The fifth coumarin having a hydroxylation pattern atypical of C_6(B)-rings is fraxinol, 5,7-dimethoxy-6-hydroxycoumarin (78).

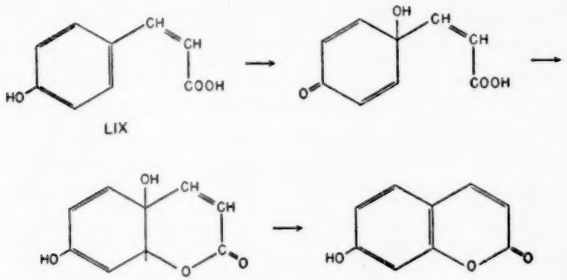
The distribution of the coumarins and the related propenylbenzenes, which are almost exclusively of the C_6(B)-ring hydroxylation type, also appears to be significant. The families in which coumarins are predominant also abound in the less complex C_6-C_3-structures, suggesting a fairly close biogenetic relationship between the two classes of compounds. These considerations indicate that the origin of the coumarins, at least of the simple hydroxylated derivatives, is similar to that of the C_6(B)-C_3-moiety of the flavonoid compounds discussed previously.

It is not difficult to conjecture how the —CHOH—CHOH—CHO side-chain of a precursor such as XXIII might be transformed to the α,β-unsaturated acid moiety necessary for coumarin formation, by a sequence of steps involving the elimination of water, reduction and oxidation of the carbonyl group. One possible route is as follows:

$$\begin{aligned} -CHOH-CHOH-CHO &\xrightarrow{-H_2O} -C(OH)=CH-CHO \xrightarrow{+2H} -CH(OH)-CH_2-CHO \\ &\xrightarrow{-H_2O} -CH=CH-CHO \xrightarrow{-2H} -CH=CHCOOH \end{aligned} \quad (69)$$

The presence of a hydroxyl group in the 4-position of the coumarin, ammoresinol (76), suggests that the terminal carbon may be oxidized and undergo cyclization prior to the reduction and second dehydration steps. This is an isolated instance, however, and can hardly serve as the basis for a proposal involving the biogenesis of coumarins in general.

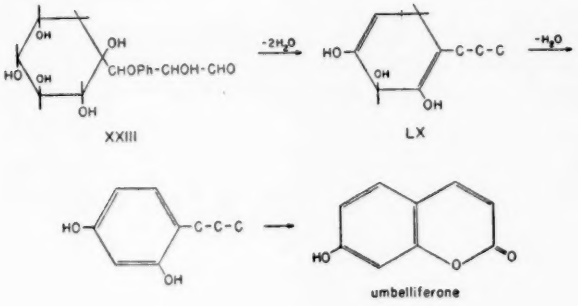
The origin of the ortho hydroxyl group which forms the alcoholic part of the lactone is less readily explained. As mentioned previously, hydroxylation in the ortho position is uncommon among C_6-C_3-compounds other than the coumarins, or in the B-ring of flavonoid substances. Haworth (1942) has suggested a route for coumarin synthesis by an oxidation and cyclization patterned after Raper's (1927) proposal for the conversion of tyrosine to melanin (70):



(70)

In his brief note no attempt is made to explain the origin of *p*-hydroxycinnamic acid (LIX) in the plant, nor does he suggest how the many hydroxylation patterns observed in the coumarin series are derived. It is true, as we have seen, that hydroxycinnamic acids and possible precursors of hydroxy-substituted cinnamic acids abound in nature, but a number of the substitution patterns common among the coumarins are not represented in the known C_6-C_3-alcohols, acids, carbonyl compounds and propenyl benzenes. The almost universal occurrence of a 7-hydroxyl group in the naturally-occurring coumarins is in harmony with Haworth's theory, but there appears to be little or no real evidence of other kinds for the proposal.

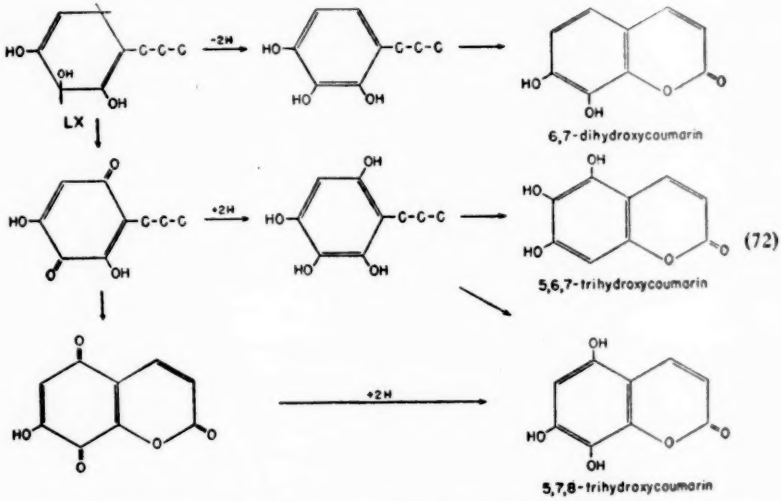
Starting with the precursor (XXIII) previously proposed as the compound from which the C_6(B)-C_3-moiety of the flavonoid compounds may be derived, it is possible to suggest reasonable routes for the development of virtually all of the basic hydroxylated coumarin structures. For example, umbelliferone could arise by a series of dehydration steps, accompanied at some stage in the process by the transformation of the hydroxyaldehyde-type C_3-side-chain to an unsaturated acid or its equivalent, as described above (equation 69).



(71)

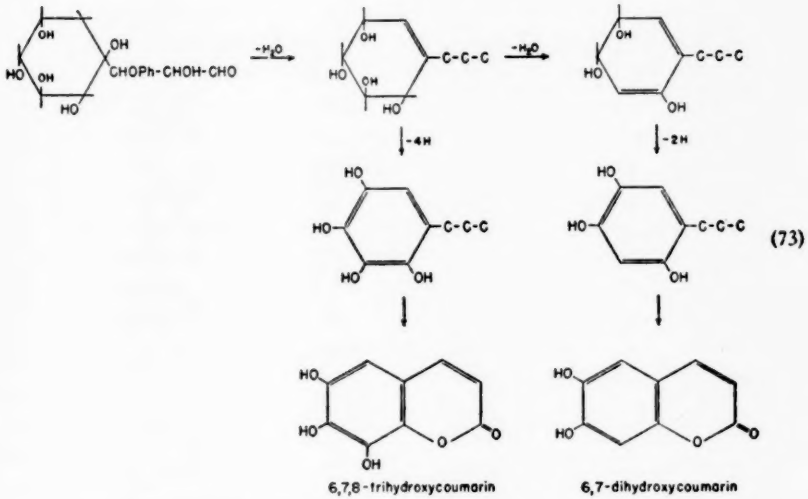
This scheme assumes an arbitrary sequence of dehydration steps, but one that is at least possible on stereochemical grounds and is consistent with assumptions used elsewhere in this section.

If, instead of the third dehydration, a mole of hydrogen is eliminated between the two saturated carbon atoms of LX to complete the ring aromatization, a 6,7-dihydroxycoumarin of the type exemplified by daphnin (72) would result. Oxidation of the partially aromatized ring to a quinone, followed by reduction to the quinol, and cyclization with one or the other of the two ortho hydroxyl groups can account for the formation of a 5,6,7-trihydroxycoumarin such as fraxinol (78) or pimpinellin (100), on the one hand, or a 5,7,8-trihydroxycoumarin structure of the sort represented by phellopterin (95), isopimpinellin (92) or byakangelicin (94), on the other hand.



(72)

The origin of 6,7-dihydroxy- and 6,7,8-trihydroxycoumarins is also explicable on the basis of arbitrary, but biochemically plausible, sequences of dehydrations and dehydrogenations (scheme 73).

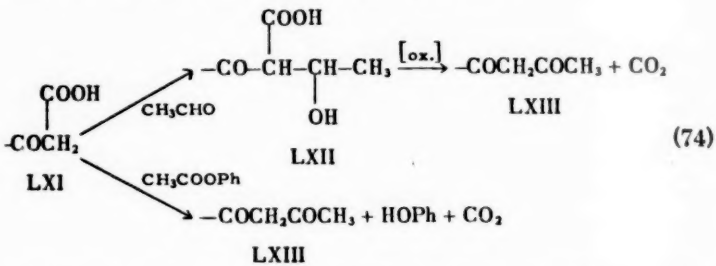


(73)

Of the hydroxylation patterns found in the known natural coumarins, including the furo- and chromano-coumarins, all are accounted for above except for the 5,7-dihydroxylated derivatives. These, though infrequent among simple coumarins, are rather common among furocoumarins where one hydroxyl group (the 7-hydroxyl in every case) forms the heterocyclic oxygen atom of the furan ring. Here too though, the occurrence of this pattern seems to be fairly well restricted to coumarins derived from a few specific plants belonging, significantly, to the Rutaceae and Umbelliferae. Indeed, all except isobergaptene (98) are found either in *Peucedanum ostruthium* or *Citrus bergamia,* although oxypeucedanin (88) and bergaptene (84) have been isolated from a few other rutaceous and umbelliferous species as well. These observations tend to strengthen the argument that the origin of the 5,7-substitution pattern in the coumarin series (and also the 2′,4′-pattern in flavonoid compounds) may be a special process inherent in the genetic make-up of a very limited group of plants. There does not seem to be any evidence at present that can give a clue to the nature of such a process. Oxidation at the methylene group of the precursor, or reduction of the 8-hydroxyl group of a 5,7,8-trihydroxycoumarin or its precursor, are explanations which can-

not be supported by analogy with known reactions. A precursor having a somewhat different structure is a possible explanation but also lacks any sort of experimental support.

CHROMONES. The similarity in basic structure, hydroxylation patterns and alkyl substituents between the coumarins and chromones suggests that these compounds have similar origins. A precursor like that proposed for the coumarins, in which the C_3-side-chain develops into a β-keto acid moiety (LXI) seems most plausible for the formation of the chromones. Subsequent condensation of the active methylene group either with acetaldehyde, followed by oxidation of the intermediate hydroxy-compounds (LXII), or with a mixed acetic-phosphoric anhydride would produce the required β-diketone structure (LXIII).

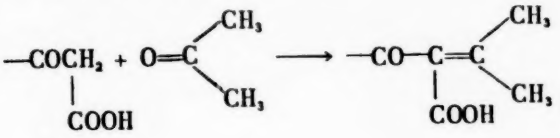


(74)

Cyclization of the labile β-diketone with an *o*-hydroxyl group would complete the chromone synthesis.

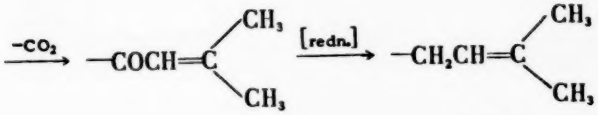
There is some evidence in the case of the chromones that the development of the side-chain occurs after the aromatic ring has been completed. If this were not so, the existence of eugenone (163), 2,4,6-trimethoxybenzoylacetone, would be difficult to explain, for, unless the hydroxyl groups ortho to the side-chain were methylated prior to the development of the side-chain into an aceto-acetyl group, ring closure to the chromone should intervene and prevent methylation of one of the ortho hydroxyls. While the above developmental sequence can be assumed only for eugenone with any degree of certainty, it appears highly probable, because of their close structural similarity, that chromones are synthesized by a similar sequence of steps.

ISOPRENOID SIDE-CHAINS, FURAN- AND CHROMANO-RINGS. Isoprenoid side-chains and derivatives based on the prenyl skeleton are encountered in most of the classes of natural products discussed in this review, and they are particularly characteristic of the coumarins. The basic isopentenyl group (LXIV) may be postulated as arising from the condensation of acetone, or a biological equivalent such as acetoacetate, with the activated methylene group of a β-keto acid or ester.



LXI

(75)

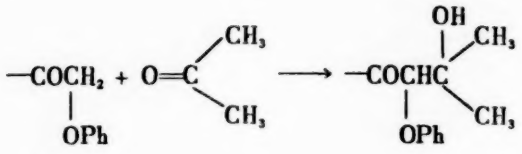


LXIV

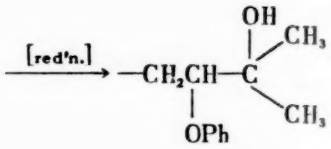
The three-carbon β-keto acid side-chain LXI could be derived originally from the common metabolic intermediate, oxaloacetic acid. The stage at which this side-chain becomes attached to the C_6-ring of the coumarin precursor, and the method by which this reaction is effected are, however, questions to which answers cannot be suggested at present.

Repeated condensations of the type outlined above may be responsible for the construction of the long, terpenoid side-chains of ostruthin (65), bergamotin (85), umbelliprenin (64) and ammoresinol (76).

Condensation of an acetone equivalent with the extremely reactive methylene group of the phosphorylated keto alcohol moiety (LXV) is another plausible scheme and would provide a precursor (LXVI) capable of developing into any of the commonly observed modifications of the isopentenyl group.



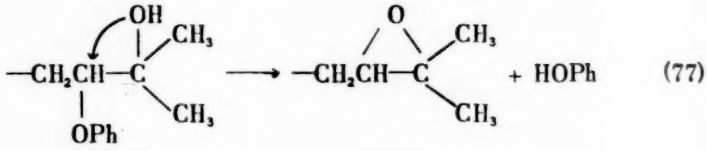
LXV



(76)

LXVI

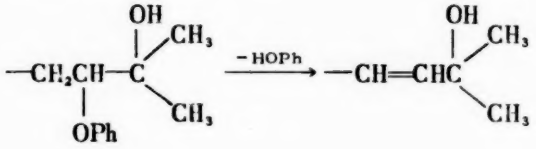
The epoxide structure (LXVII) found in auropten (68), oxypeucedanin (88), etc., may readily be visualized as arising from such a precursor:



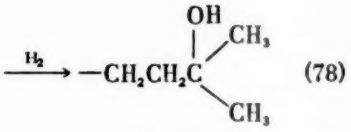
(77)

LXVI LXVII

The unique 3-hydroxy-3-methylbutyl substituent (LXVIII) of the flavonol, icariin (208), could arise by elimination of phosphoric acid from the precursor followed by reduction of the resultant double bond.



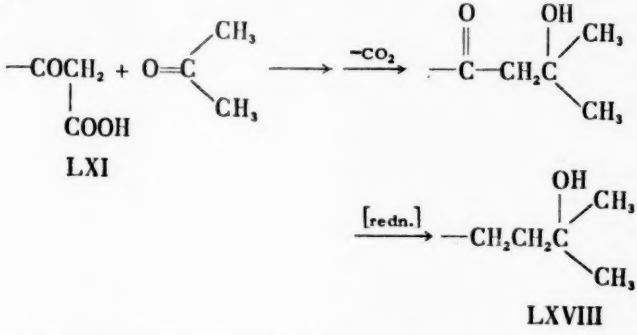
LXVI



(78)

LXVIII

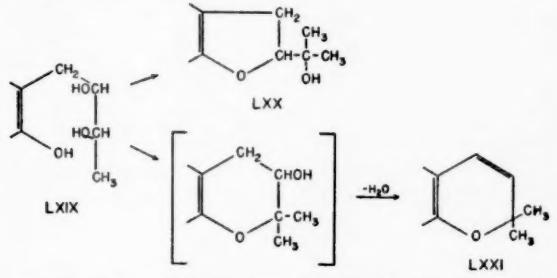
Although elimination of the tertiary hydroxyl group of LXVI would ordinarily be predicted to occur more readily than elimination of the secondary group as proposed above, the fact that this isoprenoid side-chain is attached to a C_6-ring which may have been aromatized prior to the elimination reaction could be responsible for a different result. In this case elimination of the tertiary group would produce a less stable, isolated double bond. On the other hand, the icariin side-chain could arise instead by a simple aldol condensation between acetone and the β-keto acid precursor (LXI).



(79)

Hydrolysis of the phosphorylated hydroxyl group of LXVI would account for the formation of the glycol type of isoprenoid side-chain (LXIX) found in toddalolactone (75) and ostruthol (87).

The isopropylfuran (LXX) and α,α-dimethylpyran moieties (LXXI) which are so frequently fused to the benzene rings of naturally-occurring coumarin derivatives must almost certainly result from cyclization of a prenyl group, probably as the glycol, with an adjacent phenolic hydroxyl:

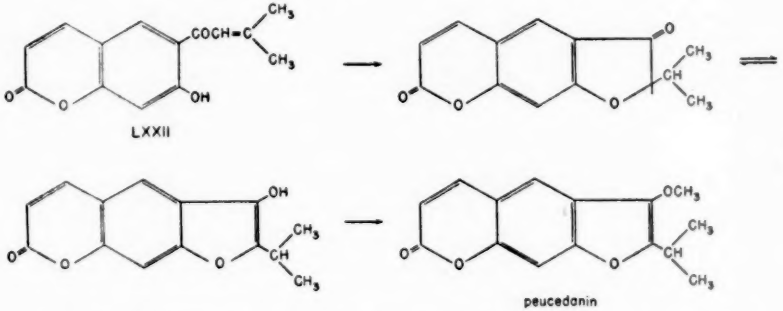


(80)

Note: The lower HOCH above LXIX should be HO-C-CH₃.

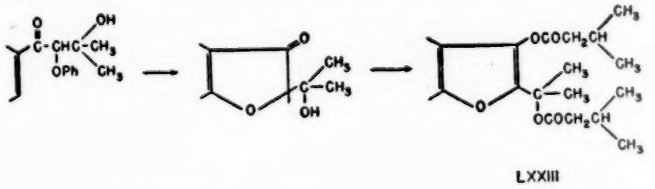
The tertiary hydroxyl group remaining when a 2-isopropylfuran ring is formed from such a glycol may remain intact, as in marmesin (96*b*), or exist in a glycosidic or ester linkage, as in nodakenin (96*a*) or athamantin (101); or it may be eliminated, producing an isopropenyl side-chain, as is found in euparin (112).

It is likely that in many cases cyclization of the five-carbon side-chain takes place before the carbonyl group of the precursor has been completely reduced to a methylene group, thus giving a furan ring with a hydroxyl group in the 3-position (equation 81). The occurrence of furan derivatives, such as peucedanin and athamantin (101), having such a substituent on the furan ring, suggests their common relationship to an antecedent, such as 6-isopentenoylumbelliferone (LXXII):



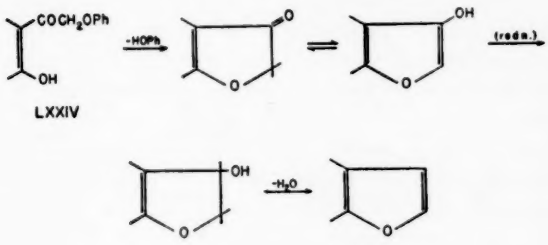
(81)

The additional hydroxyl substituent on the isopropyl group of athamantin (LXXIII) indicates further its relationship to the glycol type of isoprenoid substituent (equation 82).



(82)

Indeed the large class of furocoumarins in which the furan ring is unsubstituted could result from the two-carbon keto-alcohol precursor LXXIV in a similar manner, without preliminary condensation with acetone (equation 83).



(83)

SUMMARY

The existing theories of the biogenesis of flavonoid compounds are discussed and evaluated in terms of present-day knowledge of the chemistry of naturally-occurring compounds, plant biochemistry, enzyme chemistry and comparative biochemistry. The discussion is presented under the following topics:

1. A comprehensive summary is given of the known naturally-occurring flavonoid compounds, including substances closely related to them from the phytochemical standpoint. The classes of substances included are those having the following carbon skeletons: C_6, C_6-C_3, C_6-C_3-C_6; and subsidiary classes closely related to these.

2. The starting materials from which the complex substances of the above classes are synthesized are found in the "pool of carbon compounds" comprised of sugars, amino acids and the simple compounds arising through the interrelated processes of photosynthesis and glycolytic metabolism.

3. The synthetic mechanisms by means of which the simpler compounds found in the pool of carbon compounds are elaborated into the complex plant constituents are regarded as primarily, although perhaps not exclusively, under immediate enzyme mediation. Particular attention is paid in the discussion to those reactions by means of which carbon-carbon bonds are formed and broken: aldol and acyloin condensations, carboxylation and decarboxylation. The central role played by α-keto and β-keto carboxylic acids is emphasized. Evidence is adduced for the occurrence, however evanescent, of β-keto acids in the synthetic processes of the plant.

4. The existing theories of plant biosynthesis—in particular, of non-nitrogenous compounds—are critically reviewed. Certain of

the theories are utilized, in whole or in part, in a re-evaluation which results in the development of a hypothetical scheme of photosynthesis which rests upon a "comparative anatomy" of the known flavonoid compounds and considers the synthetic process in terms of the following discrete steps: the formation from hexose precursors of the carbocyclic C_6-fragment; the derivation of the C_3-side chain and its attachment to the C_6-fragment; the formation from a C_6-C_3 and a C_6-fragment of the basic structural unit of the flavonoid substances; the alteration of the originally non-aromatic, hexose-derived precursors into the characteristic polyhydroxybenzene derivatives of the plant substances; and the synthetic interrelationships between flavonoid compounds of different classes.

5. The questions of the mode of formation of glycosides and methyl ethers of the phenolic plant substances are discussed. Evidence is presented for the view that glycoside formation occurs late in the synthetic process. The question of the occurrence and role of formaldehyde in plant synthesis is considered in detail. The view is presented that evidence for the intervention of formaldehyde in the synthetic processes of the plant is equivocal or lacking entirely. That a "formaldehyde equivalent" is a necessary assumption is a conclusion reached on the basis of evidence of several kinds. The possibility of the intervention of the well-known methyl transferring systems of animal organisms in the synthesis of non-nitrogenous plant constituents is considered in detail. The possible role of formic acid as, or in the generation of, the "formaldehyde equivalent" is presented with the support of evidence based upon considerations of the comparative biochemistry of animal and microorganisms.

GLOSSARY

Active methylene group: a methylene group joined to a group such as an acyl group, a nitro group, etc.

Acyl: the grouping R · CO—; e.g., acetyl, CH_3CO—.

Aldol condensation: used in this paper in the generic sense, as referring to the condensation of a compound containing an active methylene group with a carbonyl compound.

Alkyl: a radical derived from a paraffin hydrocarbon; e.g., methyl, CH_3-; ethyl, C_2H_5-; etc., usually generalized as R.

Anionoid: see " Nucleophilic ". Besides the usage described there, the term may be applied to a carbon atom which may be caused to become nucleophilic as, for example, by the removal of a proton; e.g., the $—CH_2—$ grouping in a ketone, $R \cdot CH_2 \cdot CO \cdot R$.

Aryl: a radical derived from an aromatic hydrocarbon; used in this paper to refer to substituted aryl radicals as well; e.g., phenyl, C_6H_5-; *p*-methoxyphenyl, p-$CH_3O \cdot C_6H_4$-; etc.; usually generalized as Ar.

Betaine: an inner salt, or " Zwitterion ", usually a fully methylated amino acid of the type represented by " betaine " itself, $(CH_3)_3\overset{+}{N}CH_2COO^-$.

Carbocyclic: refers to cyclic compounds, the rings (cycles) of which are composed of carbon atoms only.

Carbon skeleton: the basic framework of a compound represented only by the interconnected carbon chains and rings.

Carbonyl: the grouping $—\overset{|}{C}=O$.

Carboxyl: the characteristic grouping of organic acids, —COOH.

Carboxylation, decarboxylation: the introduction or removal of carbon dioxide as represented formally by the general equation $—\overset{|}{\underset{|}{C}}H + CO_2 \rightleftharpoons —\overset{|}{\underset{|}{C}}—COOH$.

Catalytic hydrogenation: the addition of hydrogen to an unsaturated grouping with the aid of a catalyst such as platinum or nickel.

Condensation: as used in this paper the term generally refers to the coupling of two molecules with the formation of a new carbon-carbon bond.

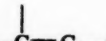
Conjugated system (conjugation): a series of alternating single and double bonds; e.g., —CH=CH—CH=CH—CH=CH—.

Cyclization: the formation of a carbocyclic or heterocyclic ring.

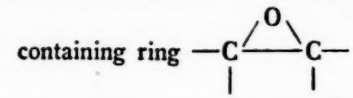
Derivative: a substance so related to another substance by modification or partial substitution as to be regarded as theoretically derived from it, even when not obtained from it in practice; thus, amines (RNH_2) are derivatives of ammonia.

Dimer, trimer, polymer: substances composed of two, three or many units of a repeating structural unit (the monomer).

Elimination reaction: a reaction typified by the removal of the elements of water from an alcohol: $RCH_2CH_2OH \rightarrow RCH = CH_2 + H_2O$.

Enol: a substance containing the grouping  .

Enolic form, enol form: the tautomeric modification of a carbonyl compound (e.g., pyruvic acid, $CH_3CO \cdot COOH$) containing the enol grouping (e.g., the enol form of pyruvic acid, $CH_2 = C(OH) \cdot COOH$).

Epoxide: a compound containing the three-membered, oxygen-containing ring 

Glycol: a compound containing two alcoholic hydroxyl groups; e.g., ethylene glycol, $CH_2OH \cdot CH_2OH$; trimethylene glycol, $CH_2OH \cdot CH_2 \cdot CH_2OH$.

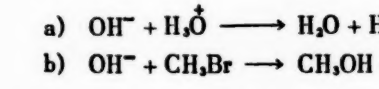
Glycolysis: the breakdown of sugars in the living organism or *in vitro* by means of enzymes.

Heterocyclic: refers to cyclic compounds, the rings of which are composed of elements of more than one kind: in this paper, usually carbon and oxygen.

Hydrogen bond: a weak bond formed between two molecules, or two parts of the same molecule, one of which contains an ionizable hydrogen atom (such as in —OH) and the other an atom possessing an unshared electron pair (such as oxygen or nitrogen).

Lactone: a cyclic ester, formed by the interaction of a carboxyl and hydroxyl group, both in the same molecule.

Nucleophilic reagent: an ion or molecule having an unshared pair of electrons and capable of reacting by donating its pair of electrons with the formation of a new bond; in many cases an atom or group and its binding pair of electrons is displaced at the same time; e.g., the nucleophilic hydroxyl group may react as follows:



a) $OH^- + H_3\dot{O} \longrightarrow H_2O + H_2O$

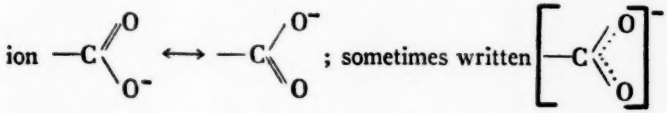
b) $OH^- + CH_3Br \longrightarrow CH_3OH + Br^-$

The term " anionoid " is often used synonymously.

Pinacol rearrangement: the reaction typified by the following example: $(CH_3)_2C(OH) \cdot C(OH)(CH_3)_2 \rightarrow (CH_3)_3C \cdot CO \cdot CH_3 + H_2O$.

Primary, secondary, tertiary: as applied to carbon atoms carrying substituent groups, refer to carbon atoms carrying two, one or no hydrogen atoms; e.g., RCH_2Br, R_2CHBr, R_3CBr.

Resonance: the concept contained in the following definition; when a substance can be represented by two or more structures which differ from one another merely by a redistribution of valency electrons of unsaturated or ionized centers, the actual molecule does not conform to any of the structures but exists as a *resonance* hybrid of them all. The double-headed arrow is commonly used to indicate resonance: e.g., the carboxylate



Substitution reaction: a reaction in which an atom or group in a molecule is replaced by another atom or group: e.g.,

$$C_6H_5 + Br_2 \rightarrow C_6H_4Br + HBr$$

Wagner-Meerwein rearrangement: a rearrangement, mechanistically related to the pinacol rearrangement, following the generation, by any of several means, of an unstable carbonium ion involving a change to one of greater stability.

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Future Contents of

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Articles Received and Awaiting Publication

Photosynthesis	C. P. Whittingham *Cambridge University, England*
Fungal Carotenoids	T. W. Goodwin *The University, Liverpool, England*
The Characeae — Their Value and Their Taxonomy	R. D. Wood *Rhode Island State College*
The Histochemical Localization of Enzymes in Vascular Plants	D. S. Van Fleet *University of Missouri*
Southern Swamps and Marshes	W. T. Penfound *University of Oklahoma*
Chemical Regulation of Sexual Processes in the Thallophytes	John R. Raper *University of Chicago*
Placentation in Angiosperms	V. Puri *Meerut College, India*
Plant Disease Forecasting	Paul R. Miller & Muriel O'Brien *U. S. Bureau of Plant Industry*
Physiological Studies on the Actinomycetes	D. Perlman *Princeton, N. J.*
Wound Healing in Higher Plants. II.	Robert Bloch *Yale University*
The Morphology of the Gynaeceum from the Viewpoint of Peltation	Hermine Baum *Universitat Wien*
Chromosome Morphology in Maize and Its Relatives. II.	A. E. Longley *U. S. Dept. of Agriculture*
Injuries to Plants Caused by Insect Toxins. II.	Walter Carter *Pineapple Research Institute*
Wood Fiber Length as Related to Position in Tree and Growth	Stephen H. Spurr *University of Minnesota*
Compression Wood in Conifers as a Morphogenetic Phenomenon	
Fossil Charophytes	Raymond E. Peck *University of Missouri*
Phylogenetic Principles of the System of Higher Plants	A. L. Takhtajan *Leningrad University*
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